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Part VI

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Proposed Guidelines for Registering Pesticides in the United States: Hazard Evaluation of Use of Pesticides for Nontarget Plants and Microorganisms

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 163 and 172

[OPP-30044; PH FRL-1545-3]

Proposed Guidelines for Registering Pesticides in the United States: Subpart J: Hazard Evaluation: Nontarget Plants and Microorganisms

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: This action proposes one subpart of the pesticide registration guidelines. Subpart J describes certain data required to support the registration of pesticide products, and deals principally with the potential damage that pesticides may inflict on desirable plants within or outside the site where pesticide applications are to be made. Guidelines in this subpart state the conditions under which specific data would be required to support applications for registratin of pesticide products. These guidelines also specify the standards for acceptable testing, provide references to test protocols in the scientific literature, and describe the formats for reporting data. The Agency believes that adherence to these guidelines would result in more reliable data to support applications. Furthermore, uniform testing and data submission in accordance with the guidelines will accelerate and upgrade the Agency's review and evaluation of applications. In addition, the Agency believes that these guidelines would aid applicants in planning the development of data to support new and improved pesticides.

DATE: Interested persons are invited to submit written comments on these proposed rules to the EPA. Comments must be received prior to the close of business on or before: February 2, 1981, and identified with the Document Control Number OPP 30044.

ADDRESS: Written views and comments should be submitted to: Document Control Officer, Office of Pesticides and Toxic Substances (TS-793), Chemical Information Division, Environmental Protection Agency, Room E447, 401 M Street S.W., Washington D.C. 20460; Attention: Pesticides.

AVAILABILITY OF SUPPORT DOCUMENTS AND COMMENTS: The support documents mentioned in this preamble and all written comments received under this notice are available for public inspection in the OTS Reading Room from 8:00 a.m. to 4:00 p.m., Monday through Friday except holidays [Room E447, 401 M Street S.W., Washington, D.C. 20460].

FOR FURTHER INFORMATION CONTACT: William Preston, Hazard Evaluation Division (TS-769), Office of Pesticides and Toxic Substances, Environmental Protection Agency, Washington, D.C. 20460; Telephone 703-557-1405.

SUPPLEMENTARY INFORMATION:

I. Authority

These reproposed guidelines are published under the authority of §§ 3(c)(2), 5(d), and 25(a) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended ("FIFRA") (86 Stat. 973; 89 Stat. 751; 7 U.S.C. 136 *et. seq.*). Section 3(c)(2) states that "The Administrator shall publish guidelines specifying the kinds of information which will be required to support the registration of a pesticide and shall revise such guidelines from time to time."

II. Background

On July 3, 1973, the Environmental Protection Agency ("EPA", "Agency") promulgated final registration regulations, 40 CFR Part 162, Subpart A. These regulations established the basic requirements for registration of pesticide products. Section 162.8 lists the kinds of data which the Agency would generally require to register a product.

On June 25, 1975, EPA published proposed Guidelines for Registering Pesticides in the United States ("1975 proposed guidelines", 40 FR 26802). The 1975 proposed guidelines were intended to describe with more specifically the kinds of data which must be submitted to satisfy the requirements of the registration regulations. They included sections explaining the scope and intent of the guidelines, detailing the product performance hazard evaluation, and chemistry data requirements for registration of a pesticide product, and providing guidance on proper label development. Some of the information now included in Subpart J regarding phytotoxicity, nontarget plant injury and spray drift appeared in the Product Performance portion of the 1975 proposed guidelines.

During the past year, the Agency has distributed prepublication drafts of Subpart J to the public in connection with reviews of this document by the FIFRA Scientific Advisory Panel. As a result, EPA received submittals of additional comments on these guidelines. The Agency has also considered these comments in the development of these proposed guidelines.

The Agency intends that the guidelines provide meaningful instruction to applicants, registrants, and the general public on the specific data requirements to accompany applications for an experimental use permit and for registration of a pesticide product. Such guidance should enable the pesticide industry to anticipate the costs and time involved in preparation and review of such applications and to plan research and testing programs using methodology acceptable to the Agency.

Toward these ends, these proposed guidelines would specify: (1) the standards for acceptable testing, stated with as much specificity as the current scientific disciplines can provide; and (2) the information required in a test report. The guidelines also indicate when an applicant should consult with the Agency before initiating certain tests. In addition, the appendix to the Subpart J guidelines provide useful information and references for designing test protocols and, in some cases, examples of acceptable protocols for conducting the required testing.

At present and until these guidelines are published as final, the procedures and data requirements for registration, the standards for acceptable testing, and the information required in a test report will be determined on a case-by-case basis. The Agency will, however, consider these proposed guidelines as a general statement of policy regarding the nature and extent of the data required to support pesticide registrations.

The Agency intends that these guidelines be revised from time to time to keep up with policy changes and technology. Revisions will be made in accordance with the Administrative Procedures Act (5 U.S.C. 551 et seq.). Under that Act, routine changes that have well-publicized precedent or minor changes that reflect no impact on policy may be made without proposal and opportunity for public comment. Changes having a significant impact on the registration process, registrants, applicants, testers, outcome and evaluation of studies, and similar related activities and affected parties, would require public notice and opportunity for comment. Until all such changes (important or minor) have been published as final rules, however, the Agency can implement them on a case-by-case basis. (For example, the Agency can use its authority on a case-by-case basis to waive certain data requirements, approve non-conforming tests, and require data from additional tests.)

. The Agency has already published four other subparts of the guidelines: B, D, and E, as proposed rules on July 10, 1978 (43 FR 29696); and F, as proposed rules on August 22, 1978 (43 FR 37336). The Agency has also published proposed rules for good laboratory practices as part of Subpart F on April 18, 1980 (45 FR 26373). Subpart B would contain general provisions applicable to all subparts of the guidelines. Subparts D, E and F would establish the requirements for product and environmental chemistry fish and wildlife toxicity data, and toxicology for human and domestic animal safety evaluation, respectively. The Agency solicits comments on any section of Subparts B, D, E, or F which would be affected by provisions contained in this proposal. Additional subparts of the guidelines will be proposed in the future, including Subpart G, Product; Performance Subpart H, Label Development; Subpart I, Experimental Use Permits; Subpart K, Exposure Data Requirements: Reentry; Subpart L, Hazard Evaluation: Nontarget Insects; Subpart M, Data Requirements for Biorational Pesticides; Subpart N, Chemistry Requirements: Environmental Fate (derived from Subpart D; will be promulgated as a final rule); Subpart O, Chemistry Requirements: Residue Chemistry; and Subpart P, Data to Support Disposal Instructions.

EPA's Office of Toxic Substances has developed testing rules under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 15 U.S.C. 2601 et seq.) which contain data requirements comparable to those proposed by section series 163.122 through 163.123 of Subpart J. The Agency's policy is to reduce the burdens on the regulated public which might arise from competing requirements under these different sets of regulations and guidelines. Therefore, the Agency will establish testing rules under TSCA and guidelines under FIFRA which are consistent to the extent permitted by the different laws.

III. Organization and Philosophy of Subpart J

Subpart J has been organized into seven series of sections. The first section series (163.120) would deal with the overview and scope of the subpart, the definitions of specific words used in the subpart, the basic standards for testing, the general evaluation and reporting requirements and the tier testing sequence policy. The second section series (163.121) would deal with target area plant toxicity testing, that is the toxicity of those plants that would be intentionally sprayed. The next four section series (163.122, 163.123, 163.124, and 163.125) would comprise the tier testing sequences (tiers 1, 2, 3 and 4, respectively) employed to study and report on pesticide toxicity nontarget area plants. The effects of the pesticides would be performed on a series of tests as dictated by specific requirements of each test and each tier. The tests would be designed to gather pesticide effects information on terrestrial and aquatic plant growth as influenced by geographical, seasonal, and species variation, on nitrogen fixation potential, and on plant mutagenicity effects. The final section series (163.126) would measure the potential for pesticides to cause plant damage by spray drift when applied by aircraft (fixed and rotary wing), mistblower (air carrier), and other application techniques. The other application techniques may include but are not limited to overhead sprinkler irrigation devices, and roadside and right-of-way sprayers.

Each individual test section would contain an opening paragraph indicating under what circumstances and for what products the data are required. These test sections would also contain specific standards and reporting requirements. In addition to these specific test standards and test requirements, the general test standards and reporting requirements would apply to the conduct of required studies.

The proposed nontarget area plant and microorganism data requirements would be arranged in a tier system. The requirement to produce data from studies in the higher tiers would depend on the results of studies in the lower tiers. The tier system is intended to reduce repetititve consultation between the registrant and the Agency about the need for tests of greater complexity, and as a result, to reduce the time required to develop data for registration of a pesticide. [Those portions of this Subpart that refer to microorganism testing other than the testing of algae may be removed to a new Subpart, yet* to be designated and developed.]

The proposed subpart would establish standards and requirements for testing and data submission concerning the effects of pesticides on nontarget plants and microorganisms. These data would be used by the Administrator, along with other information to make the determination required by FIFRA § 3(c)(5), whether a pesticide "* * * will perform its intended function without unreasonable adverse effects on the environment", and thus whether the pesticide might be registered. The proposed standards and requirements specifically concern: pesticide toxicity to desirable plants within the target area; pesticide toxicity to plants and microorganisms outside the target area, as measured through a tier system test series, and spray drift evaluation.

Specific reference to data requirements concerning hazard data is given in 40 CFR 162.18–2 (c)(2) and (d)(4) of this chapter for manufacturing-use and end-use formulated products, respectively.

The data submitted from these studies and from other unsolicited sources would be used to evaluate and draw conclusions concerning the toxicity of the pesticide to microorganisms and plants (which would include food and cover vegetation for animals, and food, fiber, timber, and ornamental plants for man). No set of results from these tests will automatically preclude the registration of a pesticide product. In certain cases, limitations, precautions, and/or restrictions would be imposed upon the use of the pesticide because of its toxicity coupled with proposed use patterns and transport characteristics.

The data requirements for this subpart are a portion of the overall environmental risk assessment. A pesticide may have a detrimental effect on several levels of production and consumption in the ecosystem. The combination of the information submitted in response to the requirements of this subpart with information derived from studies performed concerning the enviriomental fate of the pesticide, and the effect of the pesticide on fish, wildlife, invertebrates, and laboratory animals will lead to an all-encompassing risk assessment or hazard evaluation.

Concern has been expressed regarding the use which the Agency would make of data on mutagenicity and on microorganisms if such data reflected positive adverse effects. Generally, the Agency would take this adverse data into account in its overall hazard evaluation of the chemical (or its products) in relation to the proposed uses. Specifically, the Agency may, as a result of positive test results, require label restrictions or deny registration, depending on the evaluation of hazard in relation to specific use patterns. Examples of specific Agency actions regarding positive mutagenicity tests might include requirements for warnings in the instructions, such as: "Do not use for crops grown for seed production" or "Do not use except where total kill of target vegetation is intended and expected." For example of an action regarding positive test results that portend major adverse effects to certain comon soil microorganisms, a warning in the instructions might be required, such as: "Do not use in areas where soil

nitrification bacteria or mycorrhiza are important in crop production or in health of desirable ornamental plants."

A. "When Required" Paragraphs.

Each of the sections of this subpart which would establish data requirements begins with a paragraph entitled "When required." These paragraphs would estabish the conditions under which specific data would be required to support the registration of a pesticide product.

This subpart would require the necessary data to support the registration of pesticides intended for outdoor application [that which occurs outside of enclosed man-made structure(s), 40 CFR § 162.3(cc)]. There will however, be certain uses or situations where the use pattern would eliminate or markedly reduce a particular kind of risk; in such cases, the data requirements would accordingly be reduced or eliminated. For example, data from testing at higher tiers (3 and 4) normally would not be required for pesticides applied by tree injection, by directed sprayers having capture and recycling capabilities, or by incorporation into the soil. There may also be other instances in which the Agency would routinely require data to support the registration of a formulated product, even though the product was not used outdoors. For example, testing would be rquired for pesticides that are used within industrial structures where the waste water would be discharged into the environment without treatment. Finally, testing of products to be used in greenhouses would be required under the section dealing with "Target Area Plant Toxicity Testing" (§ 163.121.1).

B. "Test Standards" Paragraphs.

The proposed standards for acceptable testing are set out in the "Test Standards" paragraphs which follow the "When required" paragraphs in all of the test sections (proposed section series 163.121 through 163.126). In addition, testing would be required to be performed in accordance with the "Basic Standards for Testing", proposed § 163.120.3. The general and specific test standards for acceptable testing would identify the factors in the peffrmance of a test which EPA has determined would be necessary to ensure the reliability and completeness of the data. The test standards would cover such aspects of testing methodology as the test substance and its state, test species and life stages, number of organisms to be tested, the duration of the test, and the dosage levels.

The references provided in the test standards paragraphs or the protocols of the Appendix are recommended only.

C. Deviation from Test Standards.

The test standards are not intended to be inflexible rules or to be the exclusive methods of meeting the specified data requirements. The Agency recognizes that the prescribed standards would not be appropriate for every pesticide product. Equally effective alternative test procedures may exist and new procedures for deriving data may be developed. Accordingly in Subpart B (in § 163.40.3), the Agency proposed procedures for acceptance of data which would be derived from tests which deviate from the prescribed Agency standards (43 FR 29696, 29707, July 10, 1978).

Proposed § 163.40.3(a)(1) contains a provision governing acceptance of data which are derived form nonconforming tests: "All data required to support the registration or reregistration of a pesticide product must be derived from tests which satisfy the purpose of the standards for acceptable testing." An applicant would be required to inform the Agency whenever data have been derived from tests which do not conform to the standard. In the case of data submitted to the Agency prior to the effective date of these guidelines, the Agency may conduct this evaluation on its own initiative.

Agency files contain large amounts of data derived from tests already performed. The Agency proposes to apply the guidelines test standards to these data in a common-sense manner. To avoid expending scarce laboratory resources in repeating tests, data will not be rejected merely because they were not developed in accordance with the guidelines test standards. Rather, the Agency will accept as valid those data which permit sound scientific judgments to be made.

In addition other published and unpublished research has been conducted in the areas of seed germination, plant vigor, and spray drift that may be submitted in order to fulfill the requirements of the studies of the testing regimes proposed in these guidelines. (The term "unpublished data" means data from tests already performed but not yet submitted to the Agency or published in a scientific journal. Refer to proposed §§ 163 120.5(c) and 163.126.1(a) concerning these data substitutions.

Commenters within the Agency have questioned whether label changes could preclude certain data requirements in this subpart. Typical label instructions changed by the registration applicant are normally made in response to studies that show the product to be more hazardous than originally thought. In cases where application for registration has not yet been made, such label changes are easily made, and certain data may not be required. The Agency should be consulted when such steps are anticipated. However, label changes that reflect over-labeling (i.e., label statements warning of or implying a hazard greater than the actual hazard for such products) are not acceptable as an alternative to submittal of data. Indeed, such over-labeling would usually prompt the Agency to deny registration unless data are submitted to demonstrate how the implied hazard can reasonably be avoided.

The acceptability of data derived from nonconforming tests would depend on a number of factors. In the first place, the Agency would have to consider the characteristics of the pesticide product to determine whether an appropriate test methodology was used. Assuming that the standards for acceptable testing are appropriate for the pesticide product, the Agency would consider both the extent and the significance of the deviation and the particular test results. In general, the less significant the deviation from the standards, the more willing the Agency would be to accept data from these tests. Furthermore, the Agency would be more willing to accept data when it appears unlikely that the particular deviation would significantly affect the test results. The preamble to Subpart B contains an example and additional discussions of how these provisions would be applied (43 FR 29696, 29698).

D. Waivers and Additional Data

The amendments to FIFRA enacted on September 30, 1978, provide the Administrator with the authority to waive requirements for submittal of efficacy testing data. The Agency in testimony before Congress, stated that it is most concerned about ensuring a product's effectiveness when a lack of efficacy could result in adverse human health effects. In keeping with this concern, the Administrator has deemed that all products not having a direct impact on public health may have their efficacy requirements waived. However, under certain conditions (such as when pesticides are under cancellation or suspension, or under rebuttable presumption against cancellation), efficacy data would be required.

In the past, plant toxicity (phytotoxicity) data on desirable target area plants have been associated with efficacy data. Accordingly, data on phytotoxicity to desirable target area

plants (proposed § 163.121–1) will not be required for products with use patterns for which efficacy data requirements would also be waived.

A detailed discussion of the efficacy waiver policy with the waiver of target area phytotoxicity data requirements appeared in the preamble to the recently-published regulation on conditional registration of pesticides (44 FR 27938–27940, Friday May 11, 1979). This discussion is quoted below, in its entirety:

Section 3(c)(5) of the Act provides that the Administrator may waive data rquirements pertaining to efficacy of a product under consideration for registration, and that if he waives the requirement for data, he may also waive the finding of efficacy required by FIFRA section 3(c)(5)(A). Since efficacy data waiver is a major deregulation action, the Agency will consider the waiver policy enunciated in these regulations as an experiment in public policy. If there is sufficient evidence to clearly establish that this deregulation is being abused (such as a significant increase in complaints from the agricultural community, other user groups, or the general public about ineffective products), the Administrator may take any steps necessary to correct the abuses, including withdrawal of any or all waivers of efficacy data requirements.

The decision to pursue efficacy waiver as an Agency policy stemmed from a need to reduct the amount of resources devoted to reviewing product performance so that additional effort could be devoted to the evaluation of health and safety data, and from a desire to reduce regulatory burdens in pesticide registration. Data review obligations placed on the Agency by the requirements for registration were important factors influencing the decision. Maintenance of a rigorous efficacy data submission and review posture for registration and reregistration would require the reevaluation of much of the 1.5 million items of product performance data that have been amassed by the EPA. Because many of these studies are quite old, their value as tools for assessing current levels of product performance is questionable in many cases. Therefore, commitment of the resources needed to fully evaluate these data for purposes of reregistration was determined to be less than an ideal use of limited resources for pesticide regulation.

The Agency's viewpoint concerning waiver of efficacy data requirements is in line with a general belief among persons in the pesticide industry, the U.S. Department of Agriculture and the agricultural community that the efficacy of agricultural pesticides can be effectively regulated by the marketplace (in conjunction with extension services and university research personnel). This opinion has subsequently received some qualified support from the findings of A.D. Little Inc. (Draft Report: Evaluation Design for a Change in the Pesticide Regulatory Process: The Waiver of Efficacy Data). As originally proposed in the draft Conditional Registration regulation of October 6, 1978, the efficacy waiver was to apply to all product uses except for those termed "public health uses." These public health uses were restricted to certain disinfectant uses and a limited number of vertebrate and invertebrate control agents aimed at potential disease vectors such as bats, rats, and mosquitoes. The proposal of efficacy data waiver generated a mixed response from commenters. The pesticide industry was generally supportive of the concept of efficacy waiver. On the other hand, comments from State researchers and Cooperative Extension Service personnel expressed reservations about various facets of the proposed waiver. Many commenters expressed concern over the extent of efficacy waiver, arguing that the scope of "public health" uses should be greater than that proposed. The efficacy data requirement contained in § 162.18–2 retains the concept of public health uses, but has clarified and expanded public health uses to include additional disinfectant, rodenticide and insecticide uses, and one fungicide use.

Some commenters expressed the belief that waiver of efficacy data requirements will signal an open season to unscrupulous "fly-by-night" operators who will defraud the public by marketing ineffective products, or products with exaggerated or unwarranted efficacy claims on labeling and in advertising. Undoubtedly, there will be instances in which the marketplace will fail to operate as expected. The Agency believes that foreseeable imperfections in marketplace self-regulation are acceptable tradeoffs in return for a reduction of overall Federal regulation in the efficacy area.

EPA, in cooperation with the Experimental Technology Incentives Program of the National Bureau of Standards will monitor the impacts of the efficacy waiver policy to determine if:

(1) Consumer fraud increase[s]; or

(2) Consumer fraud is effectively reported and curtailed through existing market institutions including State regulation processes, USDA and the Extension Service, farmers associations and the marketplace.

The Agency expects that all registrants will perform the tasks necessary to assure themselves that the products the market will perform their intended functions when applied in accordance with label directions and commonly accepted pest control practices. The Agency must rely upon the integrity of the industry, the soundness of extension service recommendations and the good judgment of pesticide users to insure that abuses seldom occur. The Administrator reserves the right to request submission of efficacy data in support of label claims for to request submission of efficacy data in support of label claims for any registered product. A request may be made for any product for which a pattern of inadequate performance has been reported.

Some respondents feared that efficacy data waivers would lead to a lack of sound information on the "benefits" side of the risk/benefit analyses performed as part of the Rebuttable Presumption Against Registration (RPAR) or cancellation processes. Benefits analysis in the RPAR process entail substantially more than reconsideration of efficacy data supporting the original registration. Concern in the benefits analysis is not with historical but with current product performance. In addition, economic factors pertaining to the current use of the pesticide and efficacy of alternative chemicals or non-chemical control practices must be considered. Front-end efficacy review does not provide useful information on the suitability of alternatives. However, when a substantial risk of unreasonable adverse effects has been identified via the RPAR or cancellation/suspension processes for old use patterns of a pesticide, it is incumbent upon the Agency to more carefully scrutinize new or added uses of the pesticide, weighing those risks with the benefits they offer. Thus, the waiver policy has been amended to include a requirement for efficacy data for new or added uses of products which have been RPAR'd, cancelled or suspended for adverse effects of other uses, if the identified adverse effects would also be expected to occur with the new or added use.

A. The Agency is limiting its direct concern to, and requiring efficacy data for health-related use patterns and new and added uses of chemicals which have been identified as posing a risk of unreasonable adverse effects. Specifically, the Agency will require product performance data in support of registration for the following uses:

1. *Public health uses.* A public health use exists whenever the continued presence of the target pest organisms may pose a threat to human health, either by direct action or through transmittal of disease. Such uses include, but are not limited to:

a. *Disinfectant uses.* Performance data will be required for all antimicrobial products intended to control microorganisms infectious to man in any area (inanimate surface) where these microorganisms may present a health hazard.

b. *Fungicide uses.* Performance data will be required for products intended for control of organisms that produce aflatoxins.

c. *Invertebrate uses.* Performance data will be required for: (1) Invertebrate control products intended for use in or on humans (or in or on pets for control of pests which attack humans) to control pests such as fleas, mites, lice, ticks, biting flies, and mosquitoes. (2) Invertebrate control products intended for use either in premises or in the environment to control pests of sanitary or public health significance such as mosquitoes, biting flies, ticks, fleas, houseflies, cockroaches, fire ants, hornets, wasps, poisonous spiders, scorpions, centipedes, and bedbugs.

d. *Rodenticide uses.* Performance data will be required for: (1) Commensal rat and mouse products. (2) Products used to control or disperse birds from buildings, roosts and other areas where they present health hazards. (3) Products used to control rabies vectors such as bats, skunks, raccoons and canids. (4) Products used to control rodents considered to be significant plague vectors.

2. New uses of pesticides containing active ingredients some uses of which have been:

a. Suspended, or cancelled by the Agency for reasons of human or environmental safety (if the risks identified in the suspension/cancellation action also apply to the new use).

b. The subject of a Notice of Rebuttable Presumption Against Registration (if the risks identified in the RPAR notice also apply to the new or added use).

If efficacy data are required, the applicant may in all cases satisfy the requirement by submitting data developed in accordance with the Registration Guidelines. In some cases, the applicant may choose instead to cite all applicable data in Agency files, in accordance with §§ 162.9–1 through 162.9–8.

For disinfectant products and bait formulations or rodenticides, efficacy data, when required, must be developed and submitted for the individual formulation proposed by the applicant. The applicant may not rely on, and need not offer to pay compensaton for, data already in our files pertaining to similar formulations. It has been the Agency's experience borne out by efficacy data submitted in the past and by enforcement actions, that efficacy data in the disinfectant and rodenticide bait areas cannot be transferred from one formulation to another, even though the formulations may be virtually identical. Small variations in manufacturing process, order of addition of ingredients, or quality and age of ingredients in bait formulations appear to radically affect the ability of the product to perform as intended. Thus each individual product must be supported by its own efficacy data.

B. Efficacy data will generally not be required for products of the following types:

1. Nearly all agricultural pesticide products (including forest and crop uses). Exceptions include products falling into category A.2. above;

2. Disinfectants: Products used to control bacteria producing odor or deterioration and spoilage of products such as paint, industrial fluids, leather, where product failure does not have human health consequences;

3. Algaecides and slimicides, herbicides, fungicides, nematicides, and rodenticides except as provided in A above. Several commenters in the public meeting requested clarification as to whether phytotxicity data are included within the meaning of efficacy data. The Agency takes the position that data on phytotoxicity to the target site, i.e., crop or plants, are part of an efficacy evaluation and are thus waived. On the other hand, data on phytotoxicity to crops or other plants that are non-target sites are considered to be hazard data and must be submitted for all products as prescribed in the Registration Guidelines (Subpart J).

Notwithstanding the waiver of efficacy data, the Agency must have sufficient information about the use patterns proposed to permit evaluation of the hazards of the product to humans and fish and wildlife. In the past, efficacy data have served the purpose of providing supplementary information to the labeling use directions, enabling the Agency to accurately assess the hazards of the proposed use, and to label and classify the product to protect the public and the environment.

If the labeling submitted with an application for conditional registration is not sufficiently detailed as to the use pattern proposed, the Agency will request more detailed information may be required to be placed on the lable in the form of use directions, precautionary statements, or use restrictions if it is deemed essential to the proper use of the pesticide, or if lack of such information could potentially result in adverse effects from use of the pesticide.

In addition to the concerns reflected in the discussion quoted above, the Agency staff have also expressed concern that the waiver policy might result in ineffective products being made available on the market and in products that carry fraudulent label claims. They pointed out that failure of such products to perform the functions claimed on the label might also require regulatory action by the Federal Trade Commission.

The Agency, acting under 40 CFR 162.8(c), can impose data requirements which are not listed in these guidelines. EPA expects that the information required by the proposed guidelines will generally be adequate for hazard assessments. Occasionally, however, the Agency may need additional data in order to make the statutorily-required findings concerning the effects of pesticides on humans or the environment. EPA will decide whether to require additional data on a case-by-case basis. The guidelines will be amended to include the additional data requirement, if it has general applicability.

E. Combined Testing.

In the interest of efficient use of test organisms, facilities, and personnel resources, the Agency encourages applicants and registrants, when possible, to fulfill multiple data requirements through utilization of combined testing. Whenever a single test is performed to satisfy two or more data requirements, all applicable test standards would have to be met. For example, the appropriate tier 1 and tier 2 tests of seed germination (§ 163.122–1 and § 163.123–1) may be combined where the pesticide (such as a herbicide) has a known or suspected phytotoxic property. Also, those organisms used in growth studies (proposed §§ 163.122–1 and –2) may be used in the biochemical studies such as the nitrogen fixation potential determination (proposed § 163.125–3). The registrant should note that the species used for testing in tiers 1 and 2 (preliminary tests) (section series 163.122 and 163.123) would not be acceptable to satisfy the data requirements of tier 3 (section series 163.124) where an expanded number of species in different taxonomic groups would be used.

F. "Data Reporting and Evaluation" Paragraphs.

The proposed data reporting requirements are included in each of the test sections. These requirements, together with the requirements of § 163.80–4 would prescribe the format and content of test data reports. Generally, the test report would be required to include a summary of test results and an evaluation of test results supported by specific detailed information.

The paragraphs specifying the required data are quite detailed and would ask for considerably more basic information than has previously been required. Some of this information would be considered "raw" data. Some comments already received point out that the Agency is requiring too much detailed data. The data required by this subpart would be the basic data necessary for making independent analyses of a test. The applicants would be required to perform the analyses and evaluation of the basic data before submittal in order to become more aware of the environmental effects of their products. EPA considers it appropriate for applicants, in addition to the Agency staff, to perform this work in order that each may make independent analysis of a test.

It must be noted that data concerning equipment and certain environmental measurements used in the evaluation will not necessarily have a direct bearing on the label statements. These equipment and environmental data are necessary for the Agency to compare different studies and to obtain a more complete understanding of the various testing situations.

After the final guidelines of this subpart become effective, EPA will not accept any data which are not in conformity with the intent of the reporting requirements. However, this policy will become effective only after the passage of a reasonable time period following the effective date of the final guidelines. This time period will be specified in the final guidelines. The Agency welcomes comments on a time period that would be reasonable. Until the final guidelines become effective, the Agency strongly encourages applicants and registrants to follow the reporting requirements in the proposed guidelines.

G. Appendix.

This proposed subpart contains a single appendix which provides acceptable protocols and recommended references to other suggested protocols. Some of the proposed protocols are combinations of several methods

provided in the literature which are currently the most acceptable methodology for that specific test. Other protocols provided in the proposed appendix have been published and are generally acceptable test procedures in use by private and governmental organizations.

IV. The Standards for Acceptable Testing

A discussion of the test standards governing the methodologies of this subpart is provided below.

A. Test Substances.

The "test substance" paragraphs prescribe what chemical element, compound, or mixture(s) would have to be tested to generate the required data. One of the following substances would be required to be tested in a particular study: the technical or purer grade of each active ingredient in the product; or (one or more) typical formulated products; or the applicant's formulated product. The terms "manufacturing-use product" and "formulated product" are defined in § 163.40–2 (c) and (d) of the proposed guidelines (43 FR 29696, 29707), and were previously discussed in this preamble. The term "technical grade of the active ingredient" is also defined in § 163.40–2(a) of Subpart B of the proposed guidelines (43 FR 29696, 29707). 'Technical grade of the active ingredient" or "technical chemical" refers to a certain level of purity in the active ingredient of a particular chemical substance. Presently, the purity of the technical chemical is not fixed by an absolute standard. It is usually determined by traditional industry standards, and usually refers to that identifiable stable substance produced during the normal manufacturing process which contains the highest concentration of the active ingredient.

In all tests, the composition of the test substance would have to be precisely identified. When the required test substance is a manufacturing-use product or formulated product, its composition would also have to conform to the limits certified in accordance with § 163.61–6 of the proposed Product Chemistry guidelines (Subpart D) (43 FR 29696, 29707). The purpose and uses of certified limits are discussed in more depth in the preamble to that subpart (43 FR 29696, 29700).

In most field studies, the formulated product would be tested, since this is the substance that will be intentionally introduced into the environment. The formulated product contains one or more pesticidally-active ingredients and usually one or more inert ingredients. The inert ingredients in the formulated product may affect the placement of the active ingredient on or into the plant and the subsequent absorption by the plant. Therefore, the inert ingredients may alter the phytotoxic nature or selectivity of the pesticide.

When the technical or purer grade of the active ingredient would be tested (such as in greenhouse, laboratory, and first tier studies), other substances may have to be added to modify its solubility, dilution, or stability. This would require the use of both negative and intermediate controls to test the effect of these added substances. Testing of the technical or purer grade along with the formulated product would demonstrate effects caused by possible synergism or other interaction.

The Agency proposes that the technical or purer grade of each active ingredient in a product is the test substance used in tier 1 and 2 tests. The proposal also provides that an applicant could satisfy the data requirements for tier 1 and tier 2 tests by providing comparable data which the applicant had already produced or published in scientific journals. The Agency notes, however, that such data must be derived from studies using the technical or purer grade of an active ingredient in the applicant's product.

The Agency invites public comment on whether the laboratory and field studies should be performed using the technical or purer grade of the active ingredient(s) or the formulated product.

B. Test Species.

The test standards would specify which species of plant or microorganism must be used in the test. The species have been selected to fulfill a number of criteria specific to the organism: widely acceptable usage; existence of acceptable test methodology; economic or biological importance; and the appropriateness of its representation within the taxonomic and/or physiological group of plants to which it belongs.

Ten plant species would be required for use in the proposed seed germination and vegetative vigor studies (§§ 163.122–1 and 163.123–1). Five of these are from the basic set of plants recommended by the Franklin Institute Research Laboratories, Philadelphia, Pa., in a report to EPA (EPA 560/5–75–008). The five species are corn, oats, ryegrass, cucumber, and tomato. A sixth recommended species, green bean, would be replaced with soybean because the latter is a better indicator of soil-influencing nitrogen fixation, and its smaller seed volume would provide a more rapid indication of detrimental effects. Lettuce and cabbage have been added because both are very sensitive to chemical injury and are representative of groups that are sensitive to various environmental pollutants. Common onion has been added as a representative of an economically important and often very sensitive monocot group, the lily family. Carrot was added as a dicot representative root crop which can be directly affected by edaphic conditions and by soil-applied pesticides. By increasing the number of plants to be tested, a better indication of selective phytotoxicity would be possible, and would lead to a more accurate hazard evaluation.

Other factors influencing species selection were seed size and research utilization. The size of the seed is recognized as being directly related to the extent of phytotoxicity expression. A relatively small endosperm or nutrient supply forces the young plant to derive its nutrient more quickly from the surrounding environment; therefore, it may be affected more quickly by pesticides in the environment. Seven of the ten species required have relatively small seeds. The required species have also been utilized by a large number of researchers both as test organisms and teaching subjects to demonstrate the effects of various environmental factors on seed germination and vegetative vigor.

For the proposed aquatic plant growth studies (§§ 163.122–1 and 163.123–2), the required plant species are a duckweed and four species of algae (three freshwater and one marine). The duckweed, *Lemna gibba* G3, has been extensively used as an aquatic pollution test organism and is recommended by leading researchers in this field as the *Lemna* species to be used in these tests. The widely-used *Lemna minor* was not chosen because in too many instances *Lemna* plants have been mistakenly presumed to be *L. minor* or possibly a species of the closely-related *Spirodela*.

The four algal species represent a small cross section of a very large and diversified plant grouping. Three species, *Anabaena flos-aquae*, *Selenastrum capricornutum*, and *Skeletonema costatum* have been used in the development of acceptable algal assay bottle tests to determine the effects of pollutants on both freshwater and marine algae. The National Eutrophication Research Program, EPA, Corvallis, OR, and the Environmental Research Laboratory (for oceanic research), EPA, Gulf Breeze, FL, have selected these organisms as widely accepted representatives. In addition,

each of the species has certain strengths as a test species. *Skeletonema* is a marine diatom. It is a sensitive indicator species of aquatic pollution [Patrick, R., and C. W. Reimer. 1966. The diatoms of the United States. Vol. 1. Monographs of the Acad. Nat. Sci. Phila. (No. 13), Philadelphia, Pa. 19103]. *Anabaena* is included not only to represent the blue-green algae, but also to serve as the second indicator species of nitrogen fixation potential. *Selenastrum* has been selected by the EPA Laboratory in Corvallis as the test organism for green algae because it is nonmotile, sometimes colonial, cosmopolitan, and is easily counted by mechanical means. Testing using these aquatic species should provide a good indication of possible adverse pesticidal effects on aquatic plants.

A fourth algal species, *Navicula seminulum*, was selected as a representative of a freshwater diatom. However, it is no longer available for use, and an appropriate replacement is being sought. Scale field testing is required (tiers 3 and 4), the number of plant species would be increased to include representatives of other taxonomic groups. More species would be tested in the given taxonomic groups to determine if there is any pattern of sensitivity. Those plants of the less familiar groups would be tested because of their ecological importance. These plants are primarily the cryptogams which include the mosses, liverworts, and ferns.

The plant species proposed for testing mutagenic effects are those that have been extensively used in laboratory tests and for which the mutagenic expressions are known and easily recognizable.

Anabaena flos-aquae and the soybean *Rhizobium* symbioses have been chosen for the proposed initial nitrogen fixation potential determination. They represent two different types of organism systems capable of nitrogen fixation. *Anabaena flos-aquae* is a free-living, autotrophic, blue-green alga, and soybean contains the symbiotic, heterotrophic, nitrogen-fixing bacterium, *Rhizobium japonicum*. When an indication occurs that both plant species suffer from a lack of fixed nitrogen, the guidelines would require that the nitrogen fixation potential of a larger number of plants be evaluated more directly. *Azolla* with its nitrogen-fixing phycobiont, *Anabaena azollae*, would be required for the nitrogen fixation study due to its increasing importance as a green fertilizer for rice production in the U.S. (California) and worldwide. *Azotobacter vinelandii*, a free-living bacterium, is a major component of the soil nitrogen fixers. The method used to test nitrogen fixation potential would be the widely accepted acetylene reduction method which measures ethylene production by gas chromatography. Testing this group of organisms for nitrogen fixation potential will provide data that would allow EPA to assess the effects of pesticides on any combination of nitrogen fixers, free-living and symbionts, autotrophs and heterotrophs. The nitrogen fixation tests required by this subpart should be coordinated with those required in proposed § 163.62–8(f)(3) of Subpart D.

The selection of species for testing of pesticides is being coordinated with the Interagency Regulatory Liaison Group (IRLG) and the group within the Office of Toxic Substances working on the Toxic Substances Control Act (TSCA).

The Agency invites public comment on the selection of test species for the various tests.

C. Selection of Dose Levels.

The first level of tier testing would be required to be conducted with a dose level equal to 3 times the maximum application rate. Such levels of exposure may occur in several different situations: for example, overlapping of applications, abnormal spray drift patterns, misapplications, and extended residual activity through repeat applications. The Agency has detected numerous situations involving commonly recognized practice where the actual dosage level has exceeded the maximum recommended label rate by 2 or 3 times. By testing at the 3-fold level of the maximum recommended label rate, these situations may be assessed.

The test standards in subsequent tiers would require testing with lower concentrations of the test substance to determine the 10 and 50 percent detrimental effect levels and to ascertain a no-observed-effect level. There may be instances where responses to the calculated 10 percent and no-observed-effect levels are the same or cannot be distinguished. The concentration levels should be a progression, either geometric or logarithmic, to facilitate the determination of these detrimental effect levels. The levels should be of such a factor (about 2 for geometric progressions) as to facilitate statistical determinations.

The Agency recognizes that, in order to satisfy these standards, applicants may find it useful to perform a range-finding test before the full scale study. The Agency also recognizes that these preliminary tests can yield other information helpful to the design of the full scale study.

D. Control Groups.

In testing the formulated products, only negative control groups and the experimental groups would be required. However, when testing would involve the technical grade of the active ingredient, a second control group will be needed to ascertain the effects of added "insert ingredients" or adjuvants including solubilizers, carriers, driers, penetrators, and similar materials that are needed in order to work with the test substance. This second "intermediate" control group would be identical to the standard or negative control group but would be treated with the "inert ingredients" of the proposed pesticide product to determine any possible synergistic (or antagonistic) effects.

V. Reporting Requirements

The proposed reporting requirements for each proposed study of this subpart are separated into two sections. Proposed § 163.120–4 would establish reporting requirements that would generally apply to all testing conducted under Subpart J. The individual testing sections would establish specific reporting requirements tailored to present the results of each different kind of test. Those requirements for target area plants are given in proposed § 163.121–1 and include situations, applications, and locations where phytotoxicity is likely to occur with outdoor and greenhouse applications. Reporting requirements for the nontarget area plants are found in the individual tier sections (section series 163.122 through 163.125), and would include reporting such information as: the species, the response, and the statistical analysis. The reporting requirements for the proposed special tests of spray drift evaluation (section series 163.126) call for reporting of the equipment, methodology, and various other factors that may influence spray drift.

VI. Progression Requirements of the Tier System

The proposed tests for evaluating toxicity to nontarget area plants and microorganisms are arranged in a tier or hierarchical system. The requirements to develop data from the higher tiers would depend on the results of tests in the lower tiers. At the end of each proposed tier test section of this subpart are descriptions of the kinds of results which would result in the requirement of additional data. The tier system is arranged in a way that the applicant or registrant can develop all of the data

necessary to allow a comprehensive and complete evaluation of the hazards to nontarget plants and microorganisms. Also, the "When required" paragraph of each of the higher tiers of testing restates the proposed requirement for that test based on the results of the lower tier studies.

Comments already received question the use of EC10 as a criterion for progression from tier 1 to tier 2 and from tier 2 to tier 3 or 4. The comments state that this criterion is too stringent. EPA recognizes that certain crops can, at times, suffer a significant reduction ih growth without any effect on yield, but that a detrimental effect to growth or appearance of certain other crops such as ornamentals is generally considered unacceptable. Therefore, the EC10 was selected as the criterion.

The EC50 value for progression in the aquatic plant studies was selected based on information concerning the population dynamics of these and other aquatic organisms. Some comments received have stated that an EC95 is sufficient for the progression criteria especially with respect to algae while others refute this statement on the grounds of natural population dynamics.

For both terrestrial and aquatic plant testing, the Agency invites comments concerning the EC value system as the criterion for tier progression and also whether different standards for food and fiber crops and for ornamentals and for aquatic plants are appropriate. The tests of the various tiers may be combined or substituted with similar ones as provided for in proposed § 163.120–5. The purpose of this is to reduce the cost of testing where similar tests from various tiers would be required. Substitution of tests with similar tests already performed would also be permitted to reduce redundant testing. In all instances the test standards should be followed and the reports developed as required.

VII. Discussion of Individual Tests

This section of the preamble presents the purpose of requiring data from each of the different kinds of tests and the major issues identified in developing the individual tests.

A. Target Area Plant Phytotoxicity Testing.

The data from testing of pesticides in the target area under actual or simulated use conditions would be used to evaluate the effect of the pesticide on the growth and development of the crop or host plant and on the yield and quality of the commodity by methods of application not prohibited on the label.

B. Seed Germination and Vegetative Vigor.

Seed germination is one of the most widely accepted means of testing the effect of a substance on growth and development of plants. The seed germination study would be required to evaluate the germination process as affected by pesticides applied before or during planting.

The vegetative vigor study is included with the seed germination study, and data derived from this study would be used to predict major vegetative growth phase. The purpose is to evaluate pesticide effects on nontarget area plants with which the pesticide may come into contact.
The vegetative vigor study is a greenhouse or field plot study.

In the case of both seed germination and vegetative vigor, pesticidal effects are assessed using both processes because nontarget effects of pesticides on plant growth and development during the area plants may be exposed to pesticides at any one stage or several stages of growth.

C. Lemna Growth and Flowering.

The *Lemna gibba* test is the only study in tiers 1 and 2 which is performed with an aquatic macrophyte. It therefore can be used to assess the effect of pesticides on a representative of a very large, diversified number of vascular plants that inhabit aquatic ecosystems. *Lemna gibba* G3, when cultured axenically as indicated in the appendix to this subpart and with the addition of EDTA (9 mg/1) and sucrose (10 g/1), can be a flowering aquatic plant. These tests with this plant are the only ones contained in the first and second tier levels which would permit assessment of the effect of a pesticide on a plant while in flower.

D. Algal Studies.

Data from the testing of aquatic plants would be used to evaluate the effect of pesticides that may be applied directly or introduced into the aquatic ecosystem by leaching, runoff, or spray drift. The species selection would allow the evaluation of both fresh water and marine aquatic ecosystems.

The test procedures for the algae have been extensively evaluated by academic, governmental, and private researchers. The studies have illustrated the consistency of testing results, using the species and conditions prescribed by scientifically competent people, regardless of where the test has been performed.

It has been suggested that the tier 1 algal test use *Selenastrum capricornutum* as the sole test species. *Anabaena, Skeletonema,* and a freshwater diatom (yet to be selected) would be used in the tier 2 testing, if necessary. This would be a financial and time-saving step. The Agency invites comments on this possible modification to the algal tier testing scheme.

E. Mutagenicity Studies.

This subpart would require studies to assess possible mutagenic effects of pesticides on plants. Mutagenicity requirements are divided into two tiers. The first tier of study (proposed § 163.122–3) would be required if a pesticide produced mutagenic effects in the testing required for human hazard evaluation (see proposed guidelines §§ 163.84–1 through –4). Positivie results in the first tier of study would lead to testing in the second tier (proposed § 163.124–3). All mutagenicity test results would be used to determine the degree to which the pesticide can produce mutagenic effects in plants.

The first tier of the mutagenicity test (§ 163.122–3) would involve testing with *Tradescantia* and observing the number of pink (mutant) staminal hair cells in the exposed organisms. At present, *Tradescantia* is being used as a monitor for possible chronic mutagenic substances in air and water. There are several other tests, including the expression of waxiness in corn pollen grains, which can be used to observe mutagenic changes in plants. Some of these and additional species and methods are provided in proposed § 163.124–3.

Pesticides may cause mutations in plants in at least three ways:

(1) These pesticide may be the direct causative agent as in the case of auxin herbicides (Tomkins, D. J., and W. F. Grant. 1976. Monitoring natural vegetation for herbicide-induced chromosomal aberrations. *Mutat. Res.* 36:73–84);

(2) The pesticide may enter into the cellular metabolism process and be altered to form a mutagenically-active chemical; or

(3) The pesticide may alter a cellular metabolite which may become a mutagenically-active chemical (Plewa, M. J., and J. M. Gentile. 1976. Mutagenicity of atrazine: Maizemicrobe bioassay. *Mutat. Res.* 38:287–292).

Comments already received have questioned EPA's need for any data on the mutagenic effects of pesticides in plants. The Agency notes that in laboratory and recently in field studies, pesticides have been found to cause abnormalities in plants which are

expression of mutagenic changes [Tomkins and Grant. 1976. (above)].

Although no substantial adverse effects on plants have been reported which can be attributed to mutagenic effects caused by pesticides, theoretically, such mutations in plants would have environmental and economic significance. For example, a pesticidally-induced mutation in a pest organism could make the pest more resistent to the controlling pesticide or, in the case of a weed, more competitive with the desirable nontarget plants. As a result of natural selection pressures, the representation of these kinds of mutations would be likely to increase in the gene pool of the species. Other mutations could make individual organisms less adapted to their environment. These mutations might be expressed in crop plants which are less tolerant to pesticides or adverse growing conditions or which are less resistant to pest attack. These latter changes generally are less likely to be perpetuated in the gene pool of the species due to natural selection pressures.

EPA expects that mutations in plants are most likely to be of concern to people who use pesticides to treat plants in flower which eventually will produce seed for future crops. At present, however, several seed producers contacted by the Agency feel that testing of pesticides at a primary level for mutagenic effects is not necessary to protect seed crop plants. They state that their tightly controlled production methodology would preclude carrying any significant yield or quality changes due to pesticidally-induced mutation(s) farther than one or two generations.

EPA requests comment on the need to evaluate the potential of pesticides to cause mutagenic effects on plants. Comments should describe the nature of the problem(s) associated with pesticidally-induced mutations in plants.

Comment already received suggest that EPA would obtain adequate information about the mutagenic potential of pesticides on plants from date required to assess mutagenic hazards to humans in proposed §§ 163.84–1 through –4 (43 FR 37336, 37388.) These comments state that if it is necessary to evaluate the mutagenic effects of pesticides on plants, the data from tests using bacteria and fungi would be adequate. EPA disagrees in part with these comments. While genetic material from bacteria, fungi, and higher plants has a similar molecular structure, there are differences between these orgamisms with respect to metabolism, cytoplasm, protective membranes, cell walls and possibly mechanisms of DNA repair. These differences might affect the ultimate incidence and expression of mutations. In summary, EPA feels that testing with higher plants would provide the most reliable data for assessing mutagenic effects in higher plants. The Agency welcomes comments on the use of bacteria and fungi mutagenicity data to assess mutagenicity hazards to higher plants.

Data from mutagenicity testing using higher plants may have a second use. EPA would examine the correlation of results from plant tests with results from bacterial, yeast, mammalian, and other studies to assess mutagenic hazards to humans and consider whether the correlations support use of plant mutagenicity data to evaluate human risks. If plant-test systems are reliable predictors of possible human risks, they should be included in the battery of tests used to assess mutagenic risks to humans. These test systems possess many advantages over other test systems. They are easily adapted to the study of actual exposure conditions including low level and long duration exposures. A recent review and workshop brought out other advantages: (1) plant chromosome structures are similar to those in mammals, including man; (2) plant responses to given mutagens are similar to those of other non-mammalian and mammalian systems; (3) regeneration is possible from single haploid and diploid cells; (4) mutagen response is sensitive with short life cycles; (5) the plants are relatively inexpensive to culture and data are easily obtained from them; and (6) *in situ* monitoring is possible [Nilan, R.A. 1978. Potential of plant genetic systems for monitoring and screening mutagens. *Environ. Health Perspectives* 27: (181–196). NIEHS Workshop on Higher Plant Systems as Monitors of Environmental Mutagens, Marineland, Florida, January, 1978].

In addition to the advantages brought out by the workshop, greater statistical reliability can be attained with plant systems than with animal systems. *Tradescantia,* the most commonly-used higher plant species, contains millions of potential observable units, staminal hairs. This compares with animal tests in which only 100 or so rodents or a few thousand *Drosophila* can be used.

It is apparent that plant studies may be a readily acceptable means of testing pesticides for mutagenic effects. Numerous chemical companies have routinely submitted data from these relatively inexpensive plant studies to the Agency for evaluation to determine some of the mutagenic effects of pesticides on organisms. The continued submission of these data is encouraged until the Agency makes a final determination whether such data are or are not required by this subpart.

The Agency also invites comments concerning the second use of higher plants studies to evaluate the mutagenic potential of pesticides on humans.

F. Field Tests.

The field tests are employed to evaluate the phytotoxic nature of the pesticide in environmental conditions and situations under which the pesticide will be used.

The increase in number of species and the geographical areas will permit the Agency to more accurately evaluate the risks associated with pesticide selectivity and the varied species susceptibilities. By increasing the number of test areas, it will be possible to evaluate the effect of different environmental variables on the toxicity of the pesticide to nontarget plants and microorganisms.

G. Sorption Studies.

The sorption study (proposed § 163.125–4) is designed to derive information concerning the effects of pesticides on aquatic plants when the pesticide is likely to be carried into the aquatic system sorbed to soil or organic matter. These pesticides normally will not readily dissolve in water, nor will they have exceptionally high soil or organic carbon sorption coefficients. The pesticides so carried could be readily transferred from the soil or organic matter carrier directly to the plant surface, root, or submersed foliage, as is the normal occurence with nutrients for plants.

The other aquatic studies (proposed § 163.122–2, 123–2, and 124–2) are designed to assess the effects of dissolved material only on aquatic plants.

Because experimentation of this nature has not been conducted with the requirement to measure the various parameters, a defined level with relation to "readily sorbed" is not available. The Agency, therefore, invites the submission of information and comments concerning this type of study and a reasonable value or range of values.

VIII. Special Tests

A special test series (§ 163.126) is proposed by this subpart of the guidelines to evaluate spray drift potential of pesticides that are to be applied by aircraft (fixed wing and helicopter), air carrier (mist blower), and other ground application equipment. Examples of such ground application

equipment include but are not limited to overhead sprinkler irrigation, and sprayers for roadsides and railroad rights-of-way.

A recent amendment to the Act states "The term 'to use any registered pesticide in a manner inconsistent with its labeling' means to use any registered pesticide in manner not permitted by the labeling: Provided, that the term shall not include. (3) employing any method of application not prohibited by the labeling under FIFRA sec. 2(ee)." Under this new interpretation, liquid or wettable powder pesticides not normally expected to be sprayed (i.e., soil-injected or -incorporated) might be sprayed on occasion without the spray application being illegal or grossly "inconsistent" with the labeling. Therefore, products formulated such that there may exist a reasonable likelihood of application by spraying would generally be subject to the requirements of the spray drift studies, §§ 163.126–1 through –4.

These guidelines would establish data requirements which primarily address hazards from swath displacement. The results from the testing would be used to develop appropriate limitations on the use of the pesticide to reduce the hazards identified. Such restrictions might include general equipment restrictions, wind velocity limitations, buffer zone establishment, and release heights.

From the definition of extended airborne displacement and from the fact that damage from such drift has been significant up to several miles from the target areas, it can be seen that protection from airborne displacement cannot always be effected by means of small buffer zones around the target area. The primary factors which could equally reduce drift hazard from both swath and extended airborne displacement are related to droplet size. Hazards from both swath and extended displacement can be reduced simultaneously when technology reaches the point where uniformly-sized and -distributed droplets are: (1) sufficiently large to reduce drift, but small and numerous enough to maintain efficacy; (2) sufficiently few to prevent uneconomical and inefficient applications; and (3) which possess sufficient integrity to maintain droplet size from formation until deposition. Until that time, it would seem that reduction of drift hazard from swath displacement can be regulated and attained through specific label restrictions, buffer zones, and precautions, while reduction of drift hazard from extended airborne displacement must rely upon adherence to a combination of general label precautions and awareness by those applying pesticides of potential hazards in a particular area.

The evaluation of swath displacement is a three-part study (see proposed § 163.126–1). The first part is a bioassay study (proposed § 163.126–2) to determine the phytotoxic levels of the pesticide on plants that may be grown in close proximity to areas of application. The bioassay test performed in tiers 1 and 2, vegetative vigor (proposed §§ 163.122–1 and 123–1), may be substituted for the plant activity spectrum test. The protocols for this bioassay test are those given in the appendix: vegetative vigor (foliar application). Data to fulfill the requirement of the plant activity spectrum may be substituted by submission of published or unpublished information.

The second part of the spray drift evaluation would be the droplet volume spectrum study under proposed § 163.126–3. Inclusion of this set of data is of great importance. Droplet sizes along with wind speed are the primary determining factors of spray drift. A wind tunnel or similar device can simulate to some extent the wind conditions that may occur in the field, and can be used to study the effect of various nozzle types and cores, pressures, angles, product formulations, and droplet distribution from nozzle to impact. It should be noted, however, that the wind tunnel studies are not intended to be total substitutes for the field studies.

Because it is impossible to test all possible nozzle types and utilize all expected weather conditions, the droplet size spectrum studies would be performed using a good cross-sample of commonly-used or label-recommended nozzle types and arrangements under typical weather conditions expected during application. Equipment and conditions that simulate "worst-case" conditions for spray drift should be evaluated. The registrant or applicant would be required to determine the droplet size and volume distributions for each set of conditions. By performing these relatively less expensive tests, the number of the more expensive field studies can be reduced, and predictions of spray drift patterns under various conditions can be made.

The third part of this evaluation, the actual field determination of swath displacement spray drift using commercial equipment (proposed § 163.126–4), would be required when the plant activity spectrum indicates that plants outside the target area are likely to be detrimentally affected. Data from the field test would provide the Agency with information from actual situations and allow a more accruate hazard evaluation.

To fulfill the requirements of the droplet spectrum study and the swath displacement spray drift study, registration applicants may either conduct the studies according to the standards set forth in these guidelines, or submit published information regarding droplet size distribution and spray drift patterns which would be expected to be similar to those of the prospective pesticide product. When published information is submitted, it would have to be supported by a rationale concerning the claim of "similar or identical" spectra and patterns. While most registration applicants would probably choose to supply published data, a few may choose to develop their own data by following the standards set forth in these guidelines, simply because published information would not support the superior or different characteristics that have been incorporated into the formulations planned to be marketed.

IX. Regulatory Impact Analysis

Although individual sections of the guidelines may not be "significant" regulations under Executive Order 12044, EPA has decided to pursue the following procedure because of its concern over the total impacts of the guidelines. EPA issued an economic impact analysis of the proposed guidelines as they existed in mid–1978 published in the **Federal Register** of September 6, 1978 (43 FR 39644). As sections are reproposed or promulgated, or as new sections are proposed, EPA will estimate or revise its estimates of the costs for the relevant sections of the guidelines. The costs for these sections would be set into the context of the cost of the overall guidelines. Reference would be made to the economic impact of the total guidelines by summarizing the impacts estimated in the 1978 analysis. Once a significant proportion of the guidelines has either been reproposed or promulgated, EPA will make at least one further revision, once the guidelines are substantially complete.

A paper entitled "Economic Impact Analysis of Pesticide Registration Guidelines for Subparts G, I, and J" contains the cost and context information for the FIFRA guidelines Subpart J. This analysis is available by contacting the Document Control Officer at the address given above. The Agency

welcomes public comment on this report.

In summary, the projected total costs of reregistration for Subpart J total approximately $6 million. This amount represents a small percentage, about 0.6%, of the total data requirement costs of pesticide registration.

EPA's economic impact analysis of the proposed guidelines of 1978 was based on Subparts B, D, E, and F proposed in July and August of that year. The annualized total for registration and reregistration was estimated then at $76.5 million, some 33% lower than the current estimate of $140 million. Based on the 1978 estimates, EPA made the following conclusions about the economic impact of the whole set of registration guidelines:

1. Sales value of pesticides at the basic producers level would increase by between 1.4 and 4.4 percent.

2. Pesticide research and development expenditures would increase by between 20 and 63 percent.

3. Pesticide users would face about a 1.3% increase in pesticide costs.

4. Overall added consumer costs are estimated to be quite small (about 35 cents per capita per year).

5. Employment dislocations of 38 to 75 jobs per year are expected to be largely offset by continued industry growth.

6. The guidelines are expected to have little impact on the economic viability of the roughly 200 major active ingredients.

7. Of the 300 to 550 smaller volume active ingredients subject to reregistrtion during the 1980's, however, the guidelines were estimated to cause about 35% to become uneconomical unless significant waivers of data requirements are requested and granted.

8. The "minor use problem" [i.e., how to register or maintain registration of products for minor agricultural (and other) uses] is not expected to be significantly aggravated by the guidelines, provided a minor use policy [utilizing the USDA/State Inter-Regional Project No. 4 ("IR–4"), other existing programs, and new efforts] can be developed by EPA.

9. Major pesticide firms are not expected to be adversely affected by these guidelines. A major impact on competition in the industry, however, consists of the guidelines operating as barriers and making it more difficult for smaller firms to compete effectively. This impact may be lessened as a result of the implementation of the 1978 amendments to FIFRA.

10. The effects on pesticide formulators are not fully known at this time, and are currently being examined by the Agency through its own investigations and through contract studies.

11. There is a possibility that resource constraints (laboratory facilities and personnel) may hinder the implementation of the testing guidelines. The extent of that potential problem is currently unresolved and is being investigated.

No attempt has been made to relate Subpart J to the economic impacts summarized above.

Note.—The Environmental Protection Agency has determined that Subpart J, in and of itself, does not contain a major proposal requiring preparation of an inflation impact statement under Executive Order 11821 and OMB Circular A–107.

Dated: October 28, 1980.

Douglas M. Costle,

Administrator, Environmental Protection Agency.

Addendum I to Preamble

FIFRA section 25(a)(2)(A) requires the Administrator to provide the Secretary of Agriculture with a copy of any proposed regulation at least 60 days prior to signing it for publication in the **Federal Register.** This provision is to permit the Secretary opportunity to comment on the proposal, and he did so for the guidelines and preamble for Subparts G, I, and J. The comments of the Secretary and the Administrator's response thereto are published below. In this instance, correspondence was exchanged between Edwin L. Johnson, Deputy Assistant Administrator for Pesticide Programs, EPA, and Robert C. Riley for Barry R. Flamm, Director, Office of Environmental Quality. Each of the individual comments from USDA on Subpart J is reproduced in full, but USDA comments on Subpart G and I (also included in the letter) were not included here. The page numbers mentioned in USDA comments refer to pagination in the June 22 draft being reviewed at the time. For purposes of brevity in this addendum, each USDA comment is followed immediately by insertion of the corresponding EPA response set off in brackets.

Department of Agriculture,
Office of the Secretary,
Washington, D.C., August 6, 1979.
Mr. Edwin L. Johnson (TS–766),
Deputy Assistant Administrator for Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C.

Dear Mr. Johnson: Thank you for your July 5, 1979 letter, transmitting copies of EPA's proposed "Guidelines for Registering Pesticides in the United States." The guidelines transmitted include Subpart G—Product Performance; Subpart I—Experimental Use Permits; and Subpart J—Hazard Evaluation: Nontarget Plant and Microorganisms.

We are pleased that the U.S. Environmental Protection Agency is making progress in the development of guidelines for registering pesticides in the United States. These guidelines will be of value to those wishing to pursue registration of pesticides.

USDA cursory comments on each of the subparts are attached. We expect to have additional comments during the 90-day comment period after the guidelines are published in the **Federal Register.**

We waive the 30-day waiting period requirement for publication in the **Federal Register** after receipt of USDA comments as you requested in your letter.

We hope you will find our comments constructive. We look forward to continued cooperation with the U.S. Environmental Protection Agency as the development of these important registration guidelines progress.

Sincerely,

Robert C. Riley (for)

Barry R. Flamm,

Director, Office of Environmental Quality.

USDA Comments on Draft of Subpart J

Subpart J—Hazard Evaluation: Nontarget Plants and Microorganisms

I. Organization and Philosophy of Subpart J

This section should emphasize only essential information to fit the basic needs of regulation. *Mutagenicity studies with plants* (163.84 and 163.124–3) do not appear essential and it is recommended that the requirements be deleted.

[EPA response: The proposed tests described in §§ 163.122–3 and 163.124–3 provide that, if chemicals cause mutagenic changes in animal and microorganism systems as determined through testing under Subpart F (§§ 163.84–1 through –4), the chemicals should also be tested to determine if they also adversely affect plant systems. The Agency is concerned about the possible adverse effects on plants of mutagenic chemicals that are or may be marketed as pesticides, as discussed in the preamble at part V. E. EPA assumes that the Department has considered the Agency's reasons for proposing mutagenicity testing requirements, and that the Department disagrees with them. The Department's comment will be considered along those supplied by the public on the appropriateness, scientific merit, and feasibility of the requirements, and on the criterion that invokes the requirements.]

Pages 7 and 8—The topic of overlabeling needs further clarification to avoid confusion or improper regulation.

[EPA response: The preamble, at part I. C., paragraph 5, indicated that overlabeling (label statements implying hazard far in excess of actual hazard) of

pesticide products is not an acceptable alternative to the submittal of hazard evaluation data. The original statement has been modified slightly to clarify meaning and intent. A more detailed discussion with regard to overlabeling problems and prohibitions will be set forth in Subpart H "Label Development".]

[II. The Standards for Acceptable Testing]

Page 14—It would seem logical that EPA should only be concerned with laboratory and field phytotoxicity studies of the formulated products. There appears to be no reason to require data on unformulated components not intended for use in the environment.

[EPA response: Nontarget plant toxicity testing, as required by tiers 1 and 2 (§§ 163.122–1 and –2, 163.123–1 and –2) employs the technical or purer grade of the active ingredient ("technical chemical") for several reasons. The information on plant response to the technical chemical is sufficiently indicative of the phytotoxic nature of the chemical for preliminary evaluation, and is far less costly to develop than to require individual tests on each and every pesticide formulation. When phytotoxic effects are found, further testing at tiers 3 and 4 (§§ 163.124 and 162.125) may be required, in which case field testing employing the formulated product is performed. Since the technical chemical is the required test substance in the first 2 tiers of testing, data from already-performed tests with the technical chemical and published (or unpublished) reports of tests with the technical chemical can be substituted for actual testing by the applicant and submitted as support for the registration.] Page 18—It should be pointed out that nitrification has been addressed previously in the guidelines. Is there a need to duplicate on p. 18?

[EPA response: Nitrification (the process in which the oxidative state of nitrogen is increased) is not addressed in Subpart J. Nitrogen fixation (the process in which dinitrogen's oxidative state is reduced to produce ammonium ions) is addressed in § 163.125–3 and discussed in the preamble at part II. B., paragraph 8. The nitrogen fixation studies required by this subpart are to be coordinated with the nitrogen fixation requirements of § 163.62–8(f)(3) of Subpart D (Environmental Chemistry). Statements have been added in both the preamble and the guidelines to prevent, to the extent possible, duplication of testing.]

[V. Discussion of Individual Tests]

Page 23—The seed germination and vegetative vigor section is too broad and lacks definition. This seems to be a section concerning primarily herbicides. Soil type, moisture, content of soil, pH and other variables contribute to difficulty of interpreting laboratory, green house or early field test studies.

[EPA response: The discussion regarding seed germination and vegetative vigor in the preamble at part V. B., paragrahs 1 and 2, was modified to explain better the scope of the required studies and the intended use of the data.]

Page 25—We do not understand the relationship of plant mutagenicity testing, human hazard and practical regulation. We recommend that plant mutagenicity testing be deleted from the regulation. [EPA response: See the EPA response to the first comment in this Addendum.]

[VI. Special Tests]

Page 31 (Special Studies—Section Series 163.126)—USDA is concerned about the relationship of spray drift potential as presented in Subpart J and the need for an interagency task force between the EPA and Department of Agriculture to develop application methodology and pesticide labeling directed toward an advisory opinion relative to the "misuse" provisions concerning dilution (concentration) and unspecified methods of application. It would seem that our knowledge of spray drift as related to formulation and equipment needs greater study and followup recommendations before this part of Subpart J is accepted in final form.

[EPA response: One of the major causes of nontarget plant damage is drift of pesticides applied as sprays to areas outside the intended application site. The Agency proposes to establish data requirements to evaluate the potential of a product to drift when applied as a spray. Since these guidelines will not be published in final form for many months, there is opportunity for more information to be developed which would help the Agency provide more specific testing standards and data requirements for spray drift studies. The Agency published an advisory statement in March 1979 concerning the "misuse" of a chemical where its application concentration may not be other than indicated on the label. This opinion was provided in response to FIFRA § 2(ee). Presently, the Agency and the Department of Agriculture are involved in developing a second advisory statement on the "misuse" provision concerning dilution (concentration) and unspecified methods of application.]

General Information and Testing Requirements

The general principle of tier testing is considered acceptable but there is concern about cost if all tests are required on a pesticide product.

[EPA response: An economic impact analysis with regard to this subpart is published with these proposed guidelines. It should be noted that there are several statements in the guidelines indicating that data based on the testing requirements may be substituted with data from tests already performed (§§ 163.120–5(c) and 163.126–1(a)). Also, applicants may request that certain testing requirements be waived (see preamble at part I. D., paragraph 1), thereby reducing the costs of testing.]

Page 3—USDA is concerned about the lack of clarity in discussing target area phytotoxicity data requirements. It would seem that target area phytotoxicity studies would be generated by the States testing the product prior to developing recommendations for the farmers of the State.

[EPA response: The concern about lack of clarity is not sufficiently precise to prompt the Agency to make any changes to clarify the requirements of this subpart. With reference to the latter comment, phytotoxicity information generated by the States would definitely benefit farmers and certain other pesticide users. However, such State-generated information usually would not be available at the time EPA needed to decide whether to register a particular pesticide product. Accordingly, the burden of producing target area phytotoxicity data (when such are required) usually falls on the registration applicant.]

Page 14(3)—Maintaining plants under their normal or natural cultural environment will be impossible for many microorganisms.

[EPA response: The comment refers to § 163.120–3(e)(4) which states that test organisms "should be maintained under conditions similar to their natural or normal cultural environment." The Agency recognizes the validity of the Department's comment, but calls attention to the fact that this statement actually is only advisory in nature: note that organisms "should" (not "shall") be maintained under conditions "similar to" (not "exactly like") those natural conditions.]

Page 21—Detrimental or adverse effects to be reported do not include "effects on root systems."

[EPA response: The Agency concedes that this was an important oversight in the draft supplied to the Department for review. In response to the comment, the following wording has been added, in § 163.120–4(c)(3): "Effect on root growth and structure".]

Page 22(8)—Addressing pesticide effects on nontarget areas, other than nontarget plants, seems repetitious since other Subparts of the Guidelines cover the subject.

[EPA response: The language of the statement referred to (§ 163.120–4(c)(8) has been changed to reflect the intent of the requirement more clearly, such that duplicative requirements are no longer implied.]

[Tier 1 Testing]

Page 41—Section 163.122–1(1)—The formulated product should be the "test substance" rather than the technical chemical.

[EPA response: See the EPA response to the third comment on Subpart J in this Addendum.]

Addendum II to Preamble

FIFRA section 25(a)(2)(A) requires the Administrator to provide the FIFRA Scientific Advisory Panel a copy of any proposed regulation at least 60 days prior to signing it for publication in the **Federal Register**. This provision is to permit the Panel opportunity to comment on the impact on health and the environment of the proposal. The specific comments of the Panel on a June 22, 1979 draft of Subpart J and the Administrator's responses thereto are published below. In this instance, correspondence was exchanged between Edwin Johnson, Deputy Assistant Administrator for Pesticide Programs, and Wade Fowler, Executive Secretary of the Panel. In the letter from the Panel provided in this addendum, deletions were made that referred to the Good Laboratory Practice proposal that was reviewed at the same time. Also, readers should note that the Panel also reviewed Subparts G and I at this time; the Panel's specific comments on these 2 subparts are also deleted. For purposes of brevity in this addendum, each Panel comment on Subpart J is followed immediately by insertion of the corresponding EPA response and set off in brackets.

United States Environmental Protection Agency

Office of Toxic Substances.

Washington, D.C. 20460, October 22, 1979.

Subject: Review of Proposed Guidelines for Registering Pesticides in the United States

From: Dr. H. Wade Fowler, Jr., Executive Secretary, FIFRA Scientific Advisory Panel (TS–766).

To: Deputy Assistant Administrator for Pesticide Programs (TS–766).

The FIFRA Scientific Advisory Panel has completed review of Subparts . . G, I, and J of the Guidelines for Registering Pesticides in the United States. The review was completed in open meetings held in Arlington, Virginia, during the period July 19–20, 1979. Attached is a report of findings by the Panel.

This report was delayed due to a shift of office resources for resolution of Panel business relating to conclusion of the RPAR's on 2,4,5–T and Silvex. The secretariat regrets the delay and sincerely hopes that the delay did not significantly impede progress with proposed rulemaking on the Guidelines.

Please convey our special thanks to Dr. Preston, Mr. Jordan, and all members of the EPA staff who participated in the meeting for an excellent briefing on the important features of the proposed rulemaking documents.

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel; Review of Proposed Guidelines for Registering Pesticides in the United States

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel completed review of several additional Subparts of Proposed Guidelines for Registering Pesticides in the United States during open meeting held in Arlington, Virginia, during the period July 19–20, 1979. The following specific subparts were reviewed during the meeting:

1. ***
2. Subpart G. Product Performance.
3. Subpart I. Experimental Use Permits.
4. Subpart J. Hazard Evaluation: Nontarget Plants and Microorganisms.

Maximum public participation has always been encouraged at all meetings of the FIFRA Scientific Advisory Panel. In respect to this session of the Panel, a **Federal Register** Notice was published on July 3, 1979. In addition, telephonic notices and special mailings were sent to the general public who had previously expressed an interest in activities of the Panel. Written statements relative to the Proposed Guidelines, Subpart G, Product Performance, were received from Huntington Laboratories, Inc. In addition to comments by EPA staff, informal comments were received from the general public and representatives of the pesticide industry.

The superb efforts of Dr. William H. Preston, Jr. and other technical staff of EPA who presented various Subparts of the Guidelines to the Panel are worthy of special recognition. The Panel wishes to commend the Agency for being exceptionally cooperative in their working relationship with the Panel in all matters dealing with the proposed Guidelines. This has materially aided in the expeditious review of a large amount of technical material. Additionally, the Panel wishes to recognize the value of preliminary review of massive documents such as Subpart G, Product Performance (676 pages). Introduction of the document to the Panel and the public in Houston, Texas, during a special subcommittee meeting in November 1978 made it possible to focus attention on a limited number of central issues and avoid lost time in discussions emanating from problems of communication relative to isues easily resolved by Agency officials.

In consideration of all matters brought out in the meeting and a careful review of the proposed rulemaking document * * *, the Panel submits the following report:

The FIFRA Scientific Advisory Panel is of the opinion that the *** subparts of the Guidelines (Subparts *** G, I, and J) submitted to the Panel as proposed rulemaking during the Twenty-fourth meeting of the Panel, July 19–20, 1979, deals effectively with the intended procedures with a limited number of specific exceptions and recommendations:

The FIFRA Scientific Advisory Panel unanimouslyu submits the following comments on the *** proposed subparts of the pesticide registration Guidelines:

I. ***

II. ***

III. ***

IV. Subpart J Hazard Evaluation: Nontarget Plants and Microorganisms.

This subpart encompasses concerns relative to potential damage to plants within the target area that are intended to be protected as well as valuable plants outside target areas. Panel Comments and Recommendations: The Scientific Advisory Panel concurs with this subpart with the stipulation that the Agency will provide appropriate remedies to a series of minor issues identified and recorded by EPA staff during the meeting.

[EPA response: The more important of the minor issues noted and acted upon by the Agency include:

(1) Preamble, part II. B., paragraph 2: The statement mentioning statistical analysis between species has been deleted.

(2) Preamble, part IV., paragraph 6: A paragraph has been added regarding testing of combinations of nozzles and nozzle configurations. It explains that studies should be performed using a good cross sample of commonly-used or label-recommended nozzle types and

arrangements used under typical weather conditions, and that, during field evaluations, the commonly-used equipment and expected weather conditions that provide "worst case" conditions should be used.

(3) § 163.120–3(e) and § 163.120–4 (throughout): The repetitious wording "nontarget plant and microorganism test species" has been changed to "test organism".

(4) § 163.120–3(f): A paragraph on nontarget organism safety has been added to help ensure that endangered or threatened plant and animal species will not be adversely affected by conducting the field tests required by this subpart.

(5) § 163.120–4(a): A statement has been added to reflect the need to obtain an experimental use permit for field studies where more than 10 acres are involved or where spray drift studies are to be performed.

(6) §§ 163.122 and 163.123: The statement under "Test substance" referring to use of water as a diluent has been deleted.

(7) § 163.125–3: Cross reference is made to § 163.62–8(f)(3) of Subpart D to prevent duplication of testing for nitrogen fixation potential effects.

(8) Appendix Item (2): A change has been made indicating that vegetative vigor studies using *Rhizobium*-inoculated soybeans should be conducted during the 7–9 week period so that the effect on *Rhizobium* nitrogen fixation can be observed.]

[End of comment.]

It is proposed to add a new Subpart J to 40 CFR Part 163 to read as follows:

Subpart J—Hazard Evaluation: Nontarget Plants and Microorganisms

General

Sec.
163.120–1 Overview.
163.120–2 Definitions.
163.120–3 Basic test standards.
163.120–4 General evaluation and reporting requirements.
163.120–5 Tier testing sequence.

Target Area Testing

163.121–1 Target area plant toxicity testing.

Tier 1 Testing

163.122–1 Seed germination/seedling emergence and vegetative vigor (Tier 1).
163.122–2 Growth and reproduction of aquatic plants (Tier 1).
163.122–3 Mutagenicity effects (Tier 1).

Tier 2 Testing

163.123–1 Seed germination/seedling emergence and vegetative vigor (Tier 2).
163.123–2 Growth and reproduction of aquatic plants (Tier 2).

Tier 3 Testing

163.124–1 Terrestrial field testing (Tier 3).
163.124–2 Aquatic field testing (Tier 3).
163.124–3 Mutagenicity testing (Tier 3).

Tier 4 Testing

163.125–1 Widespread geographical uses (Tier 4).
163.125–2 Widespread seasonal uses (Tier 4).
163.125–3 Nitrogen fixation potential (Tier 4).
163.125–4 Readily sorbed materials (Tier 4).

Special Testing

163.126–1 Spray drift: General requirements.
163.126–2 Spray drift: Plant activity spectrum studies.
163.126–3 Spray drift: Droplet size spectrum studies.
163.126–4 Spray dreft: Field evaluation.
Appendix A to subpart J

Authority: Secs. 3, 8, and 25(a)(1), Federal Insecticide, Fungicide, and Rodenticide Act, as amended; 92 Stat. 819; (7 U.S.C. 136 et seq.; 1972, 1975, and 1978).

General

§ 163.120–1 Overview

(a) *General*—(1) *Scope.* This subpart deals with data requirements to support registration of all outdoor use pesticides that come in contact with plants and microorganisms. This subpart addresses testing for adverse effects to nontarget plants, including those which are within the pesticide application target area (such as crop plants that are growing with weeds or are hosts for insecs and disease organisms), those which are outside the target area (such as typical adjacent crop plants, desirable ornamentals, important wildlife food and cover species, and forestry, lumber, and conservation plantings and endangered and threatened plant species), and microorganisms both within and outside of the target area. This subpart is concerned with toxicity resulting from direct exposure (i.e., application of a pesticide to a plant or microorganism) and from indirect exposure (i.e., exposure resulting from movement of the pesticide through the environment).

(2) *Organization.* This subpart contains three broad areas of data requirements:

(i) Toxicity to plants in the target area (§ 163.121–1);

(ii) Toxicity to plants and microorganisms outside of the target area (section series 163.122, 163.123, 163.124, 163.125); and

(ii) Evaluation of spray drift potential (section series 163.126). These data must be derived from tests and reported in a manner which complies with the general test standards contained in § 163.120–3 and the general reporting requirements contained in § 163.120–4 as well as the specific standards and reporting requirements of each section listed in paragraphs (a)(2)(i) and (ii) of this section.

(b) *Waiver of requirements for target area phytotoxicity*—(1) Section 3 of FIFRA requires an applicant for registration of a pesticide to submit data showing that the product "will perform its intended function without (causing) unreasonable adverse effects on the environment." Toxicity data are necessary to show that products do not cause undue damage to nontarget organisms, including plants, in the environment. However, in accordance with Section 3(c)(5) of the amended FIFRA, "(i)n considering an application for the registration of a pesticide, the Administrator may waive date requirements pertaining to efficacy, in which event the Administrator may register the pesticide without determining that the presticide's composition is such as to warrant proposed claims of efficacy." The Administrator has determined that efficacy test data include target area phytotoxicity testing data, and that data submittal for such testing may ordinarily be waived for most kinds of pesticide products. Such products generally include all pesticides whose uses result in direct or indirect application to plants in the target area such as agricultural, lawn, and garden use. Target area phytotoxicity data submittal will not be waived for pesticides that are under review in a cancellation or suspension proceeding, or aginst which a rebuttable presumption against registration (RPAR) notice has been issued.

(2) Even though the Administrator will ordinarily waive the requirement for submittal of target area phytoxicity test data as indicated in paragraph (b)(1) of this section, he reserves the authority to require such data on a case-by-case basis whenever he deems that such data are necessary to evaluate the acceptability of a product for registration. Thus the guidelines in this subpart shall be used by registration applicants as phytotoxicity test standards and phytotoxicity data reporting requiremetns when target area phytotoxicity data must be submitted to support registration applications. The guidelines may also be used to provide helpful guidance on testing to support the claims and directions for use on product labeling for products for which target area phytotoxicity data submittal is waived.

(c) *Nontarget area phytotoxicity testing.* Data concerning the determination of pesticidal effects on nontarget area plants and microorganisms required to support the registration of pesticide products for outdoor applications are arranged in a tier system. Each tier test section

specifies the conditions under which the data are required. Tests in the lower tiers (1 and 2) are designed to screen those technical chemicals to determine the potential to cause adverse effects on seed germination, vegetation vigor, and aquatic plant growth and reproduction. The higher tiers (3 and 4) are designed to broaden knowledge concerning any determination effects on nontarget plants and microorganisms of either technical chemicals or formulated products. Waivers of specific nontarget phytotoxicity test data may be requested. The request for waiver must address the product applicaiton methodology, the pesticide product's chemical and physical properties, and the known phytotoxicity properties of the pesticide product.

(d) *Pesticide movement that relates to phytotoxicity evaluations.* During and after pesticide application, pesticides may be transported through the environment to nontarget areas where they may induce undesirable effects on nontarget plants. For some uses and some pesticides, data may be required concerning transport by wind (via spray drift, volatilization), water (via leaching, lateral movement in soil, runoff, drainage), movement of contaminated soil itself, and movement of treated plant material. The spray drift evaluation guidelines of section series 163.126 will not deal with other types of pesticide aerial movement such as violatization and movement by wind of treated or contaminated soil or plant material.

(e) *Application rates in relation to phytotoxicity evaluations.* Application rates for these tests generally include stated on product labeling, but may also include higher concentrations or more frequent applications that label-recommended rates to cover those use situations where commonly recognized practices of pesticide usage frequently involve over-application.

(f) *Comparability tests.* (1) In certain circumstances, the registrant may establish the comparability of detrimental effects between proposed and registered uses of formulations rather than conducting a large-scale testing program in which all variables pertinent to the amended use or altered formulation are extensively reevaluated. These circumstances may exist when a registrant seeks to amend his registered label by altering directions for use, rates, or applicaton techniques, or by adding tank mixes, serial applications, adjuvants, or alternate carriers, or when a registrant seeks initial registration of an alternate formulation of a product which he has previously registered. Use of this approach will generally decrease the time and expense involved in generating toxicity data in support of registration. Prior to initiating testing to establish comparability, the applicant should discuss with the Agency the propriety of this approach and the toxicity data required in the specific situation encountered. Establishing comparability requires direct comparisons of results from plots (preferably adjacent ones) subjected to identical test condition.

(2) Since tests may be fewer in number and smaller in size than those involved in full scale testing programs, more emphasis must be placed on selection of test conditions such that comparability will be evaluated under the most rigorous test conditions.

(g) *Relation of Subparts B, D, F, G, and H to Subpart J.* (1) The registration applicant is referred to Subpart B—Introduction to the Guidelines for: purpose of the guidelines, definitions of widely-used terms, policy on flexibility in relation to deviation from test standards and acceptable protocols, and requirements for retention and submission of raw data, specimens, and samples of test substances.

(2) Data required by other sections of these guidelines for pesticide registration contain information which may be considered in the evaluation of pesticidal hazards to plants resulting from the use of a pesticide. This information appears in the following section series:

Sec.
163.62 Environmental chemistry.
163.84 Mutagenicity testing.
163.90 Overview, definitions, and general requirements.
163.91 Efficacy of antimicrobial agents.
163.92 Efficacy of aquatic pest control agents.
163.93 Efficacy of fungicides and nematicides.
163.94 Efficacy of terrestrial herbicides, plant regulators, desiccants, and defoliants.
163.95 Efficacy of invertebrate control agents.
163.96 Efficacy of vertebrate control agents.

In no instance will the data requirements of these sections be imposed such that duplicative data would have to be submitted to meet the requirements of these sections and of this subpart.

(3) The registration applicant is referred to Subpart H—Label Development for requirements on pesticide labeling. One of the important objectives of the testing programs required in Subpart J is to develop sufficient data to support appropriate and adequate precautionary statements and instructions for use, with respect to nontarget plants and microorganisms. Applicants should read the appropriate paragraphs of § 163.100–4 and section series 163.104 dealing with phytotoxicity and nontarget plant effects.

§ 163.120–2 Definitions.

Terms used in this subpart shall have the meaning set forth in FIFRA at § 162.3 of this chapter, at § 163.40–2, at § 163.60–2, and at § 163.90–2. In addition, for the purposes of this subpart:

(a) The term "algae" includes all chlorophyllous *Thallophyta* other than the *Bryophyta.* It includes the blue-green algae (*Cyanophyta*), green algae (*Chlorophyta*), golden algae and diatoms (*Crysophyta*), brown algae (*Phaeophyta*), red algae (*Rhodophyta*), and golden-green algae (*Xanthophyta*).

(b) The term "aquatic plants" includes those plants which are totally aquatic (free-floating or attached, submersed, and emersed) and those which are semi-aquatic such as swamp and wetland plants.

(c) The term "drift" as defined in 40 CFR 162.3(n) means movement of a pesticide through air during or immediately after application or use to a site other than the intended site of application or use. Drift includes two major components:

(1) Swath displacement downwind: that which is deposited in or adjacent to the intended target area.

(2) Extended airborne displacement: that which is carried and deposited beyond and not necessarily adjacent to the target area.

(d) The term "ECx" means that external pesticide concentration required to cause a detrimental change or alteration (in a nontarget plant) expressed as a percent (x) in comparison to untreated control plants. An EC10, EC50, and EC90 are the concentrations required to effect a 10, 50, or 90 percent detrimental change, respectively, on nontarget plant growth or activity.

(e) The term "EDx" means that internal pesticide concentration or dosage required to detrimentally affect dry weight gain (in a nontarget plant) expressed as a percent (x) in comparison to untreated control plants.

(f) The term "Ix" means that pesticide concentration required to effect a detrimental change (usually inhibition) in enzymatic activity in a nontarget plant expressed as a percent (x) in comparison to that in untreated control plants.

(g) The term "microorganism" means any of those organisms classified as algae, fungi (*Myxomycota* and

Eumycota), and bacteria (*Schizomycota*).

(h) The terms "nontarget plant" and "nontarget microorganism" mean any plant and microorganism species considered not to be pests. These species are not intended to be controlled, injured, killed, or detrimentally affected in any way by a pesticide. "Nontarget plants" include desirable or pest host plants such as crops or ornamentals within the target area, and desirable plants outside the target area.

(i) The term "pest-free" means as free of pests as possible. For example, for herbicide tests which require pest-free plots, such plots should be free of weeds; for tests with insecticides which require pest-free plots, such plots should be free of damaging insects.

(j) The term "phytotoxicity" means unwanted detrimental deviations from the normal pattern of appearance, growth, and function of plants in response to toxic pesticides and to other toxic chemicals that may be applied with the pesticide. The phytotoxic response may occur during germination, growth, differentiation, and maturation of plants, and may be of a temporary or long-term nature. Phytotoxic responses include adverse effects on growth habit, yield, and quality of plants or their commodities to the extent that a relationship between cause and effect can be established.

(k) The term "plants" includes vascular and nonvascular plants, algae, and fungi.

(l) The term "target area" means the area actually treated with a pesticide when label use directions are followed.

(m) The term "target area plants or microorganisms" means all plants or microorganisms located within the target area, and includes both desirable and undesirable species.

§ 163.120–3 Basic test standards.

(a) *Scope.* This section contains test standards which apply to all studies in this subpart unless another section of this subpart contains a specific test standard on the same subject, in which case the specific test standard in the other section shall apply to the conduct of that particular study.

(b) *General.* The experimental design, execution of the experiments, classification of the organism, sampling, measurement, and data analysis in support of an application for registration must be accomplished by use of sound scientific techniques recognized by the scientific community. The uniformity of procedures, materials, and reporting must be maintained throughout the toxicity evaluation process. Refinements of the procedures to increase their accuracy and effectiveness of the evaluation are encouraged. When such refinements include major modifications of any test procedure or standard, the Agency should be consulted before implementation. All references supplied with respect to protocols or other test standards are provided as recommendations.

(c) *Personnel.* (1) All testing and evaluation must be done under the direction of personnel who have the education, training, and/or experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The Agency may require resumés of personnel who have supervised, performed, evaluated, or reported the testing.

(2) To help assure consistency in the development of data, one person should be responsible for each particular phase of study.

(d) *Test substance.* (1) Plant hazard evaluation tests to support the registration of a pesticide shall employ either the technical or purer grade of the active ingredient or the formulated product(s), as specified in the following series of sections in this subpart: 163.121, 163.122, 163.123, 163.124, 163.125, and 163.126.

(2) The composition of the test substance shall be determined, including the name and quantity of contaminants and impurities, as far as is technically feasible, in order to account for 100 percent of the test sample. If the test substance is a formulated product, it shall be within the limits, if any, certified in accordance with § 163.61–6(b).

(3) Samples from the same lot of the test substance shall be used throughout a particular test or study. The samples shall be stored under conditions that maintain their purity and stability. In the case of formulated products, storage should be under conditions as found in commonly-recognized storage practices. If the stability of the test substance cannot be maintained for the duration of the study or if, for other reasons, it is not possible to use the same lot throughout the test, subsequent lots of the test substance should be selected that are as nearly identical physically and chemically to the original lot as practical. Chemical assays shall be performed to assure this identity and permit reporting of any deviations in compositions.

(4) For long term studies, such as field tests, when the test substance is incorporated into or applied to soil or water, the initial concentration of test substance in the soil or water shall be determined and samples collected periodically to determine the concentrations. (This information may be cross-referenced to the data requirements of §§ 163.62–7 through 163.62–11 in Subpart D.)

(5)(i) If a carrier, vehicle, or adjuvant is used to dissolve, dilute, or modify the physical characteristics of the test substance for any study, it should be chosen to possess as many of the following characteristics as possible:

(A) It should not interfere with the metabolism of the test substance;

(B) It should not alter the chemical properties of the test substance; and

(C) At levels used in the study, it should not produce physiological or toxic effects to plants.

(ii) Products with labeling which recommends the addition of separately-packaged adjuvants to the spray tank must be tested to determine whether any detrimental effects (such as increased crop phytotoxicity) may result from their addition to the pesticides. The adjuvants to be tested for use with a pesticide are those adjuvant brand names or defined adjuvant classes specifically named on the pesticide label. If a range of adjuvant rates is recommended, the maximum and minimum rates within that range must be evaluated in conjunction with the intended pesticide product.

(6) For testing pesticides in aquatic systems, the test substance must be solubilized in the water to the extent that it will be present in the environment as though introduced by a formulated product.

(7) In addition to or in lieu of data required by this subpart, the Agency may require, after consultation with the applicant, data derived from testing to be conducted with:

(i) An analytically pure grade of an active ingredient;

(ii) The technical grade of an active ingredient;

(iii) An inert ingredient of a pesticide formulation;

(iv) A contaminant or impurity of an active of inert ingredient;

(v) A metabolite or degradation product of an active or inert ingredient;

(vi) The pesticide formulation;

(vii) Any additional substance which enhances the phytotoxic activity (up to and including synergistic effects) of the product for which registration is sought; or

(viii) Any combination of the test substances mentioned in paragraphs (c)(7)(i) through (vii) of this section.

(8) When tank mixtures are recommended on product labeling, a study must be conducted to demonstrate the extent of antagonism and synergism with respect to detrimental effects on

nontarget plants and microorganisms of the products of tank mixtures. Antagonism and synergism are best evaluated in adjacent plots where possible interactions are subjected to statistical analysis.

(9) Data requirements for serial application(s) of one or more pesticide(s) preceding or following another pesticide on the same crop area in the same growing season are identical to those described in paragraph (d)(8) of this section for tank mixes with respect to phytotoxicity, when such serial applications are recommended on the label.

(e) *Nontarget plant and microorganism test species.* (1) The organism species or groups to be tested are specified in the following series of sections of this subpart: 163.121, 163.122, 163.123, 163.124, 163.125, and 163.126.

(2) Healthy plants must be used.

(3) Either cultivated crop, ornamental, or wild indigenous plants may be used; endangered or threatened species as determined by the Endangered Species Act of 1973 (Public Law 93–205) shall not be used.

(4) Test organisms that are obtained from natural systems and which are to be used for testing should be maintained under conditions similar to their natural or normal cultural environment.

(f) *Nontarget organism safety.* While performing field tests, all necessary measures shall be taken to ensure that nontarget plants and animals, especially endangered or threatened species, will not be adversely affected either by direct hazard or by impact on food supply or food chain.

(g) *Controls.* (1) Control groups are used to assure that effects observed are associated or attributed to the test substance exposure. In phytotoxicity evaluations, all treated plots, plants, and commodities must be compared directly to untreated control plots, plants, and commodities. The appropriate control group shall be similar in every respect to the test group except for exposure to the test substance. Within a given study, all test organisms including the controls shall be from the same source. To prevent bias, a system of random assignment of the test plants to test and control groups is required. Where a carrier, vehicle, or adjuvant other than water is used, appropriate experiments and controls should be included to distinguish the possible action of the carrier, vehicle, or adjuvant.

(2) When technical chemicals are formulated with dissolving or dispersing agents to make solutions or mixtures suitable for the performance of the tests, the solutions or mixtures without the technical chemical must be included as additional controls in the test regimen.

(h) *Equipment.* (1) All equipment used in conducting the test, including equipment used to prepare and administer the test substance, and equipment to maintain and record environmental conditions, shall be of such design and capacity that tests involving this equipment can be conducted in a reliable and scientific manner. Equipment shall be inspected, cleaned, and maintained regularly, and be properly calibrated.

(2) The application equipment used in testing products in small field plot studies must be designed to simulate conventional farm equipment. This can be accomplished by using the basic components of commercial application equipment in the design of the small-plot equipment. For example, nozzle types, sizes, and arrangements on small plot sprayers can be identical to those used by growers on commercial ground sprayers; or single-row commercial granular application equipment mounted on a garden tractor for small plot trials should produce results comparable to a multiple of such units on a large tractor. For large-scale field trials, commercial application equipment must be used. Specific details as to description of equipment design, adjustment, and operation must be provided in test reports.

(i) [Reserved]

(j) *Observations.* All observed signs or absence of toxicity to plants and microorganisms and related detrimental effects must be determined and reported.

§ 163.120–4 General evaluation and reporting requirements.

(a) *General.* (1) The registration applicant is reminded that certain efficacy data submittal requirements may be waived as described generally in § 163.120–1(b) of this subpart.

(2) The registration applicant is also reminded that an experimental use permit will usually be required in order to conduct field spray drift studies described in § 163.126–4. Also, experimental use permits may be required for the testing of pesticides under field conditions involving more than 10 acres, such as in described in §§ 163.121–1, 163.124–1, 163.124–2, 163.125–1, and 163.125–2.

(3) The report shall include a detailed and accurate description of test procedures, materials, results and analysis of the data, a statement of conclusions drawn from the analysts, and a tabular summary and abstract of results. When they have been determined, the primary and secondary modes of action with respect to plant morphogenic and biochemical levels should be reported.

(4) The metric system shall be used in test reports. The U.S. standard measures also be included in field test studies. The two systems shall not be mixed (e.g., g/sq. ft.).

(5) The English language shall be used in all test reports. English translations must be provided with foreign language reports.

(b) *Test materials and methods.*—(1) *Dates.* Actual dates of the studies shall be reported, including date(s) of initiation (planting, transplanting, and cultural practices), application(s), observations, and harvest.

(2) *Laboratories.* The names of the laboratories or institutions performing the tests shall be included.

(3) *Personnel.* Name and title of each principal investigator, and the name, address, and phone number of the employer shall be reported.

(4) *Test substance.* Identification of the test substance shall be provided, including:

(i) Chemical name, molecular structure, and quantative and qualitative determination of its chemical composition;

(ii) Relevant properties of the substance tested, such as physical state, pH, and stability; and

(iii) General identification and composition of any vehicles (e.g., diluents, suspending agents, and emulsifiers) or other materials used in the testing of the substance.

(5) *Untreated control (check) plots.* Detailed descriptions of plots and plants or microorganisms used as control treatments for comparisons of toxic effects must be included for each test. Data must indicate if the control plot or plants were untreated (received no pesticide), received standard cultural practices (which must be described) similar to those for plants in the experimental plot, and received other pesticides (which must be listed). Data from untreated plots or plants must include measurements of the same factors to be evaluated in determining detrimental effects resulting from use of the proposed pesticide.

(6) *Test organisms.* The description shall include the indentification of the test organisms (genus, species, and cultivar or variety, as appropriate), rationale for selection of the species employed, and location of plant collection areas including their physiographic data. When plant species othe than those identified for specific studies have been tested, their degree of susceptibility to the pesticide must be included in the test report. This

susceptibility should be reported in terms of EC values as in the regular test plant reports.

(7) *Location.* Geographic location, including relation to the target sites, shall be reported.

(8) *Substrate conditions.* (i) For aquatic pesticide applications, the following physiographic conditions shall be reported:

(A) Type of aquatic site, such as lake, pond, reservoir, stream, or irrigation ditch with flow rate (if moving water);

(B) Size (area and depth or volume or length, width, and depth of the treated areas, and of the whole site), as is the appropriate to the type of application and the type of target organism(s);

(C) Water quality, including pH, temperature, hardness, alkalinity, salinity;

(D) Turbidity, conductivity, and dissolved oxygen (for submerged plants only); and

(E) Soil texture, including that of soils along the immediate shoreline or ditchbank and the submersed soil where the target pests are present (with the percent organic material in the soil also reported). (Recommended methods and soil texture classifications may be found in the Walkley-Black Procedures in *Soil Sci.* 63:251, 1947, and the Soil Survey Manual, U.S. Dept. Agr. Handbook No. 18, 1951, Fig. 1, and *Soil Sci. Soc. Amer.* 26:305–317, 1962.)

(ii) For terrestrial pesticide applications, the following physiographic conditions shall be included:

(A) The edaphic conditions and characterization including soil type and texture, approximate pH and temperature, and presence of a hard pan or shallow bedrock;

(B) The degree and direction of slope and its orrientation to the row direction.

(9) *Environmental conditions.* (i) For growth chambers and laboratory experimentation, the light quality, light quantity (lux), air temperature, humidity, photo- and thermoperiods, and watering schedules shall be reported.

(ii) For greenhouse and field experiments, the approximate light quantity (usually expresed in degree of cloudiness), high and low daily air temperatures, relative humidity, and photoperiod (day length) shall be reported. The field environmental conditions are required only for the day of application. Area environmental conditions may be used to satisfy long term study data requirements.

(10) *Application*—(i) *General.* The test substance application method shall be reported, including dosage rates, application equipment (nozzle, orifice, pressure), time and number of applications (with reference to season and stage of growth), spray dilution, spray volume per unit area, and adjuvants;

(ii) *Dosage rates.* Dosage rates shall be reported in units of active ingredient or acid equivalent as appropriate. Rates may be expressed as units of ingredient per unit of land area to be treated, units of concentration (such as parts per million), units per flow rate, or units of ingredient per unit volume applied to obtain a specified degree of foliage coverage (such as "to runoff"). If a product is applied more than once within a year or growing season, each rate and the interval between applications must be indicated. If products are applied in a tank mixture or are applied serially, rates and intervals, as appropriate, must be reported with identification and formulation for each product.

(iii) *Timing of applications.* When the test substance, particularly herbicides, plant regulators, desiccants, or defoliants, is applied to any desirable nontarget plants within or adjacent to the target area, the plant's stage of growth or development at application must be described in test reports.

(iv) *serial applications.* In addition to the detrimental effects of the pesticides, the times of application (or application interval) must be indicated for each product or tank mix involved in the serial application.

(c) *Observations.* (1) Observations shall be reported to include all variations, either inhibitory or stimulatory, between the treated test organisms and the untreated control test organisms. Such variations may be phytotoxic symptoms (chlorosis, necrosis, and wilting), formative (leaf and stem deformation) effects, growth and development rates, and stage of development and dates when adverse results occurred and subsided or recovered.

(2) Observations shall be reported in sufficient detail as to allow complete evaluation of the results. This evaluation, to be performed by both the registrant and the Agency, shall include the degree or extent of effects exerted by the pesticide in question for each replicate and variable.

(3) The detrimental or adverse effects to be considered and reported during the observation period of terrestrial studies shall include:

(i) Stand or plant population;

(ii) Overall vigor of the plants expressed as height, weight, diameter, length, or other similar aspect of growth;

(iii) Phytotoxicity or visible symptoms such as discoloration, malformation, desiccation, or defoliation;

(iv) Lodging of plants;

(v) Effect on root growth and structure;

(vi) Development delay or acceleration with respect to emergence, vegetative growth, flowering or sporulation, and maturation;

(vii) Yield of the crop or commodity that is affected as compared to those of crops or commodities of untreated check plots; and

(viii) The effects of pesticide applications on grass or the other desirable plant stands and growth where these plants are used to prevent erosion.

(4) Where pesticides are applied to aquatic systems and influence plant growth and development in aquatic systems, the effects of that pesticide on nontarget plants must be evaluated and reported, including vigor of the plants, phytotoxicity or other visible symptoms, and delay or acceleration with respect to vegetative growth, flowering or sporulation, and maturation.

(5) Uniform scoring procedures shall be used to evaluate the observable toxic responses.

(6) At least two methods of evaluation (such as linear and mass determinations) must be used in the evaluation of pesticide effects on growth, reproduction, and yield of plants in greenhouse and controlled chamber experiments. When direct measurements cannot be made, such as in large field evaluations, a zero to one hundred (0–100) or zero to ten (0–10) scale shall be used, where zero (0) indicates no injury and one hundred or ten (100 or 10) indicates a total kill produced by the test substance. An explanation of the steps of the rating scale employed shall be included with the report.

(7) Observation reports shall include the basic data used for the statistical analysis [see paragraph (d) of this section]. Such data shall include the actual values used to determine any percentages of effects. Raw data (chromatographs, field reports, and analysis data) may also be included to substantiate the basic data that are required.

(8) The effects of pesticides on plants in nontarget areas adjacent to the target area must be evaluated and reported. These areas may include any aquatic or terrestrial site where pesticides may drift, be carried by soil erosion or surface water runoff, or otherwise be transported by natural means.

(d) *Statistical analysis.* (1) When test results such as efficacy, phytotoxicity, or yield indicate adverse effects on crops and other nontarget test organisms, statistical analysis is

required to evaluate the response(s). The statistical analysis shall consist of:

(i) The tablulation of the response data at each treatment level;

(ii) Calculation of the slope of the dose response line;

(iii) The determination of 10, 50 and 90 percent detrimental effect levels (e.g., EC10, ED90, I50, as appropriate) and the 95 percent confidence limits for each;

(iv) A goodness of fit test of the dose response curve; and

(v) The estimated non-discernible effect level. This is the level at which there would be no significant effect on the intended yield, quality, or aesthetics of the crop or plant which might be exposed. The value reported may be either calculated (usually calculated as a 5 percent reduction in growth, yield, or quality) or observed directly.

(2) Statistical analysis is also useful in evaluation of interactions resulting from studies supporting tank mixtures or serial applications.

(e) *References.* Copies of references or literature used in modifying the test protocol, performing the test, making and interpreting observations, and compiling and evaluating the results shall be submitted. Copies of unpublished literature shall also be included. Copies of the recommended literature referenced in these guidelines are not required.

(f) *Special test requirements.* In addition to the data required in this subpart, data from other tests may be required by the Agency for making judgments regarding safety to nontarget plants. Such data will be required where there are special problems, such as a proposed pattern of use, mode of phytotoxic action, or a unique chemical property. Methods are usually derived from those already described or cited in other subparts of these guidelines.

§ 163.120–5 Tier testing sequence.

(a) *General.* The data requirements and their associated protocols in section series 163.122, 163.123,163.124, and 163.125 are arranged in a hierarchical or tier system. Data from certain basic tests will be required of pesticides for registration (tier 1, §§ 163.122–1 through 163.122–2). Additional tests will be conducted on the basis of criteria addressing certain data requirements and standards. The criteria to proceed from one tier to the next are contained in the "Tier progression" paragraph of each section. The requirements to perform a specific test or test series are also given in the "When required" paragraph of each section.

(b) *Performance.* Before a product is to be considered for registration, all tests required by the tier testing systems must be performed or referenced.

(c) *Substitutions.* If the pesticide has been extensively tested by the registrant using screening tests similar to those of tiers 1 and 2, the data from those tests may be submitted in lieu of tier 1 and 2 test data (other than mutagenicity). The term "extensively tested" means testing of at least the plants or plant families represented in §§ 163.122–1(b)(2) and 163.122–2(b)(2) under environmental conditions suitable to determine any phytotoxic effects. The reports shall be submitted as provided in paragraphs (c) of §§ 163.122–1, 122–2, 123–1, and 123–2. The Agency will reserve the right to require testing as provided in tiers 1 and 2 (exclusive of mutagenicity testing) if the submitted test data do not prove to be adequate to assess a pesticide's phytotoxic nature.

Target Area Testing

§ 163.121–1 Target area plant toxicity testing.

(a) *When required.* Data concerning the phytotoxic effects of a pesticide on desirable target area plants are required to support the registration of each formaulated product intended for outdoor and greenhouse applications. Such data submittal are waived or not waived in accordance with the policy set forth in § 163.120.1(b).

(b) *Test standards.* In addition to the general standards set forth in § 163.120–3, the target area phytotoxicity testing shall meet the following standards:

(1) *Test substance.* The formulated product shall be tested to support the registration of a formulated product.

(2) *Test species.* Those desirable target area or pest host plant species as listed on the label (for example, the crop plant or ornamental) which will be within the target area shall be tested. The plant cultivars to be tested shall include representatives of the cultivars that are most likely to be used.

(3) *Dose levels.* (i) The minimum, maximum, and 3 times the maximum label dosage levels shall be tested. Levels greater than 3 times the label rate may also be included. The estimated non-discernible effect levels shall also be determined.

(ii) The multiples of the dosage rate to be tested are those various quantities of the formulation in the label-recommented quantity of carrier-(such as water) to be used per land or aquatic use area.

(iii) Appropriate controls shall be utilized. Refer to § 163.120.4(f).

(4) *Adjuvants.* Products with labeling which allows or recommends the addition of separately-packaged adjuvants to the spray tank must be supported with data indicating any detrimental effects (such as increased crop phytotoxicity) which may result from their addition to the pesticide, especially a herbicide, plant regulator, desiccant, or defoliant. If a range of adjuvant rates is recommended, the maximum and minimum rates within that range must be evaluated in conjunction with the intended pesticide product.

(5) *Site.* The test shall be performed in crop fields or greenhouses where it is intended that the product would be used.

(6) *Protocol.* The protocols, methods, or practices shall be those employed during the actual use of the pesticide product. Specific points of information that should be addressed concerning use patterns, application methodology, cultural practices, responses, and subsequent planting are found in paragraph (c) of this section.

(c) *Reporting.* In addition to the information required by § 163.120–4, the test report shall include the following information with respect to phytotoxicity to the plants within the target area (with the exception of weeds). This information shall include the method of application, cultural practices, plant responses, subsequent plantings, and use patterns that may be involved.

(1) *General information*—(i) *Timing of applications.* When crops or desirable target area plants are or will be involved in the application of any pesticide, their stage of growth or development at application must be described in the test report.

(ii) *Meteorological conditions.* Where meteorological conditions cause detrimental effects on plants which in turn allow the pesticide to further adversely affect the plants, the specific factor(s), such as temperature, wind conditions, precipitation, or daylength, affecting product activity must be measured and reported. Edaphic factors, such as soil moisture content and temperature, which are directly affected by meteorological conditions, must also be reported. Soil moisture may be observed and expressed in terms of dry and cracked, waterlogged, or other similar conditions.

(iii) *Spray dilutions.* In foliar applications, when a pesticide is applied as a diluted spray and the quantity is dependent upon the number of trees per area or density of vegetation, any detrimental effects to the nontarget plants must be reported.

(iv) *Untreated controls (checks).* In phytotoxicity evaluations, all treated plots, plants, and/or commodities must

be compared directly to untreated control plots, plants, or commodities. All quanlity and/or yield evaluations of pesticide-treated plants or commodities must be compared to control plants or commodities receiving the same pesticides (e.g., herbicides, insecticides, fungicides) except the one being evaluated. Detailed descriptions of plots and plants used as control treatments for comparisons of detrimental side effects must be included for each test. Since such control plots are established to evaluate any direct detrimental effects of the pesticide on the crop or commodity rather than an evaluation of efficacy, any detrimental effects on the crop or commodity resulting from the pests intended to be controlled must be eliminated by controlling these pests in another manner. In other words, the control plots must be both untreated by the pesticide in question and as pest-free as possible. If, in addition to the untreated control plots, plants, and/or commodities, a registered product is also applied (as a standard) for comparison of detrimental effects, data must indicate the standard product's name, active ingredient, dosage rate, and performance results.

(2) *Use patterns.* When the following use patterns are found on the label, the corresponding information as detailed below must be reported.

(i) *Use in field crops.* Effects of pesticides on desirable target area plants must be evaluated and reported. The extent and duration of the effect must be expressed in terms of stand and vigor, recovery, yields, and degree of phytotoxicity.

(ii) *Use on pastures and rangelands.* Effects of pesticides on desirable target area plants must be evaluated and reported. Severity and duration of adverse effects on desirable plant species, expressed in terms of stand and vigor reductions, recovery, and changes in yields, must be reported. Data must be submitted addressing: Reseeding intervals which minimize adverse effects on reseeded plants, and animal grazing recommendations which allow recovery of desired plant species. Data must be submitted which describe general shifts in desirable plant populations due to treatment.

(iii) *Use on and around fruit and nut trees.* Applications of pesticides on and around fruit and nut trees require evaluation and reporting of detrimental effects on foliage, and changes in growth compared to pre-application measurements. Pesticide applications to bearing fruit and nut tree areas also require evaluation and reporting of detrimental effects on yields and commodity (produce) quality for the year of and the year after application. Supporting data must address, for all trees, the age of the trees, the transplant-to-application interval, and the maximum allowable extent of contract between the pesticide (with particular reference to herbicide spray drift) and trees. For ground sprays, unless the pesticide is broadcast over the entire orchard floor, data must indicate the application technique (band, spot, shielded, or directed spray application) and the size of the treated ground area around the tree trunk. For foliar sprays, the data must include the volume of finished spray applied per unit of land area, concentration of product in the spray solution, and the extent of foliage coverage (such as volume of finished spray per tree or application to the point of runoff).

(iv) *Use on lawns and turf.* Evaluation of effects of pesticides on representative species or cultivars of desirable lawn and turf plants should include such factors as color, density, percent cover, growth rate, rooting, and tillering. Data must address use on newly seeded lawns by demonstrating safety to representative species and cultivars of desirable lawn plants claimed, with appropriate seeding-to-application intervals. Data must address use of an appropriate application-to-reseeding interval for each desirable lawn species and variety claimed. Interactions between herbicide application and lawn cultural practices (such as raking, mowing, mowing height, watering, and fertilizing) must be evaluated for possible adverse effects on desirable lawn species. In situations where fertilizer and a pesticide are applied serially and both types of products may contact the emerged crop foliage (such as in turf or lawns), the interval between application of the pesticide and the fertilizer must be reported, as well as any resultant phytotoxic effect, stunting, or discoloration, and recovery time for the injured desirable species.

(v) *Use around ornamentals.* Phytotoxicity data in support of use on or around an ornamental or crop species must include an evaluation of the sensitivity of representative cultivars of that species. Test data must identify the method of application as to directed spray and/or topical applications. Growth stage of the ornamentals and the transplant-to-application interval (when applicable) must be indicated in the test data. Information must be submitted on specialized nursery cultural practices employed in tests, such as use of artificial soils, mulches, containerized stock, and other pesticides.

(vi) *Use in forest development.* The effects of the pesticide on desirable plant species commonly present in forest development, in addition to the desirable forest trees, must be indicated in the report with any detrimental or adverse effects that the pesticide may cause. Special attention must be given to pesticidal effects on noncompetitive ground cover species that aid in the land management practices such as erosion control. Small plot testing techniques may be used to determine the effect of pesticides on ground cover plants. Large plot testing (for example, quadrat or line interception method) should be used for pesticide evaluation in larger standing vegetation. (A recommended reference is: Phillips, E. A. 1959. Methods of Vegetation Study. Hold, Rinehart, and Winston, Inc.: New York, N.Y. 107 pp.)

(3) *Application methodology.* All methods of pesticide application not prohibited by the label [see § 2(ee) of the Act] shall be evaluated and reported. Specific detail as to descriptions of equipment design, adjustment, and operation must be provided in test reports involving aerial applications and applications using conventional farm equipment (such as tillage or planting equipment), irrigation systems, mechanical incorporation, directed sprays, subsurface placement, or band rather than broadcast distribution.

(i) *Aerial application.* Guidance and the data requirements for testing aerial applications are provided in § 163.126–1.

(ii) *Irrigation system application.* (A) For irrigation system applications, multiple plots within a treated field must be examined and the results reported from crop phytotoxicity (expressable as yield quantity and quality of harvestable commodity) as an indication of pesticide hazard. Data from such plots must be reported for each individual plot and not simply averaged together. It is important that, in addition to the standard requirements for conventional applications, submitted data must include soil texture, percent soil organic matter, relative soil moisture content (dry, medium, or wet) at application, acre-inches of water applied, and precipitation quantities within one week after application.

(B) For overhead sprinkler irrigation systems, plots must be located at several nozzle positions along the length of the lateral. Plots must be placed at both extreme ends of the lateral as well as in areas where the sprinkler patterns overlap. The concentration of active ingredient at several nozzles along the lateral must also be determined and reported. Comparison studies should be made between plots treated

conventionally with ground application equipment and irrigated with untreated water, and plots in the same field treated through the irrigation system.

(C) For surface irrigation systems, the following data are required. Concentrations of active incredients in water must be determined for the study plots where the treated water enters the field, and at the lower end of the field or where the water exits. When furrow irrigation is used, data must indicate the spatial relationship between crop rows and furrows. If the pest control in furrow irrigation applications is intended only for the furrow itself and not the bed between the furrows, the data must so indicate.

(iii) *Directed sprays.* When sprays are directed toward or away from certain portions of the soil or plants, data must indicate nozzle arrangements, nozzle orientations, the extent of spray contact with soil or plants, and application height.

(iv) *Subsurface soil applications.* When pesticides are applied directly beneath the soil surface (injected through shanks or spray blades, gravity-fed, or incorporated), test reports must include information on the application equipment. For example, for injection equipment, the following must be specified: application device spacing, depth or operation, injection pressure, speed of operation, volume of liquid or gas applied per unit area or linear row distance, and the injection device-to-row spacing orientation.

(v) *Other applications.* When a pesticide is applied to an aquatic system other than an irrigation system, the following application information must be included:

(A) Target site where the pesticide was applied (for example, to weed foliage, to surface of water, to bottom of water body, into water, to ditchbank, or to shoreline);

(B) Description of the equipment used to apply the pesticide (for example, ground-spraying device, pumping device; boat, blower, helicopter, or fixed-wing aircraft):

(C) Description of any water level changes used in conjunction with the pesticide application, such as drawdown operation or drainage of conveyance system, including the extent of water level change, the time of the change in relation to the pesticide application, and the duration of the change in water level; and

(D) The timing of the application in relation to the calendar date and the stage of growth of the target and nontarget organisms.

(4) *Cultural practices.* Cultural practices for a given use pattern or application method vary with production areas and frequently from grower to grower within an area. The effects of cultural practices on the product's possible detrimental effects must, therefore, be addressed.

(i) *Irrigation.* Irrigation and watering practices must be studied as a variable if the product is to be used in irrigated areas or greenhouses, respectively. The influence of different irrigation practices must be studied in the use area. Refer to § 163.120(c)(3)(ii) of this subpart concerning information required for irrigation practices. Irrigation data must include a description of equipment and techniques used in water application, the number and timing of irrigations, and quantity of water in acre-inches (hectare-centimeters) applied at each irrigation. Also, a description is required of the chronological relationship between irrigation applications and application of the pesticide, such as herbicide, plant regulator, desiccant, or defoliant. Where flood irrigation is utilized (such as in rice production), depth, duration, and any "flushing" must be described for each test. When irrigation is used to activate a pesticide in the absence of precipitation, the minimum and maximum application-to-irrigation interval (producing the desired efficacy level) must be reported. Since crop safety is often influenced by pesticide placement in the soil profile, and irrigation may directly affect such placement, label-recommended or allowed irrigation practices must be supported by crop safety data (phytotoxicity and yield). When irrigation practices result in loss of pesticide-contaminated water (as in runoff or drainage) from the target area, data must be submitted addressing effects of such water on nontarget plants.

(ii) *Mowing.* Mowing operations may have a direct effect on detrimental effects from pesticides intended for use on lawns, turf, golf courses, median strips, pastures, rangeland, and hay and forage crops. Mowing just prior to or just after a pesticide application may, by mechanically injuring desirable plant or by decreasing growth rates, increase injury to desirable plants (especially young shoots). Mowing just prior to application may be a requirement for plant regulators intended to maintain the neat appearance of grassy areas by retarding grass growth. In situations where mowing is routinely a part of cultural practices, or may influence detrimental effects, such practices must be reported in test results.

(5) *Target area plant responses.* The detrimental effects on crops, commodities (produce), or any other desirable plant species or commodity within the target area must be evaluated and reported. The following are some of the characteristics that should be addressed:

(i) *Stand.* Crop stand counts, reported as percentage of untreated control crop stands, must be submitted to support pesticides applied prior to crop emergence.

(ii) *Vigor.* Crop vigor (or stunt) ratings or measurements (plant height, weight, diameter, or length) in treated areas must be compared to plants in check plots in which commercially acceptable levels of pest control are maintained. Vigor ratings must be reported at the point of maximum stunting. If stunting is observed, it is important that subsequent evaluations be made to document the degree of recovery.

(iii) *Planting depths.* A range of planting depths within the range recommended for the crop must be included in preliminary studies with preplant and preemergence (to crop) applications. Date obtained from these trials must reflect any effects of varying planting depths on the incidence of crop injury that might be encountered under commercial use conditions. In subsequent trials, commercial planting equipment at recommended depth settings must be used. If in preliminary studies the planting depth is found to be a critical variable, crop emergence data must be taken from all trials.

(iv) *Loading.* The effect of pesticides on lodging of target area crops such as soybean, wheat, corn, sorghum, rice, or sugarcane must be indicated. Observed percent of treated plants affected and the severity or approximate degree of angle of lodging in treated plots must be compared to that in weed-free check plots.

(v) *Phytotoxicity.* Evaluations of visible symptoms of pesticide injury (such as discoloration, malformations, desiccation, defoliation, or death) to crop plants must be at least visually assessed and reported. These symptoms must be compared to results in untreated pest-free check plants. Evaluations must be performed at the time injury is first observed and at periodic intervals thereafter to document the degree of recovery.

(vi) *Development.* Effects of pesticides on plant development (such as delayed emergence, prolonged vegetative growth, delayed or decreased flowering or fruit set, or delayed maturation) must be indicated in test results. If such effects are outgrown by or before the usual harvest date, such recovery must be reported.

(vii) *Yields.* Effects of pesticides on yields must be reported. Yield data can confirm that there are no detrimental effects on the desirable target area plants due to the pesticide application. Yield data may also be used to evaluate benefits derived from the application. When yields are evaluated in relation to crop safety or phytotoxicity, yields from treated plots should be compared to yields from untreated plots. Comparisons of treated and untreated (control) plot yields, when expressed as weight of seed (grain and dry beans) or hay, must be based upon equivalent moisture contents (percent moisture) acceptable for commodity storage. In the case of weed control, yields from weedy check plots may be reduced as a result of weed competition and may mask crop injury due to herbicide application. Therefore, herbicide yield comparisons should be drawn from the treated plots and untreated pest-free plots. When any adverse effects indicated in paragraphs (c)(5) (i) through (vi) of this section occur, the ultimate indication of their impact can usually be evaluated at harvest.

(viii) *Crop and commodity (produce) quality.* In some cases, pesticides may not affect yields, but may affect the quality of the crop or commodity (produce) and its value. Crop and produce quality must be reported based on comparison with check plants. Flavor and acceptance studies are required for crop plants or commodities intended for direct consumption by humans and animals. Quality determinations will vary widely with crops or the uses of end products. As an example, fruit finish is important for fresh market citrus, but may be of little consequence in citrus for processing where rinds are discarded. The use of an official grading system such as USDA's is appropriate for many fruit, vegetable, and agronomic crops. A study of the quality of a fiber crop must indicate trash content and fiber length, strength, uniformity, color, condition, and diameter. For bulb, corn, and tuber crop treatments involving ornamentals, evaluations must continue for two growing cycles to determine effects on flowering. A quality study of a seed crop must indicate seedling vigor and percent germination. In cases where crops are produced for commodities (such as sugar, oil, or drugs) other than raw vegetable matter, both quantity and quality of the finished product must be evaluated. If crop or commodity quality is a factor in determining value, an optional method of indicating quality is by indicating dollar value per unit quantity of both treated and check crop or commodity. This approach is often advantageous since the grower's main concern is the monetary return for the crop. This system also does not require extensive background information on grading systems, and quality evaluations.

(6) *Subsequent planting.* The effects of pesticides on desirable plants subsequently planted in the area of application must be evaluated and reported. Subsequent planting may include emergency replanting of crops or trees within the target area where crop failure may have occurred and where the planting of rotational crops (including cover crops) takes place after the harvesting of the crop present during the pesticide application.

(i) *Emergency replanting.* If pesticide labeling states that crops may be safely replanted after an initial crop failure, the submitted data must support: the crops suitable for replanting; pesticide application-to-replanting intervals; additional pesticide applications recommended or allowed; recommended soil tillage; and soil and meteorological conditions under which replanting is or is not recommended. For example, when the original pesticide was applied in bands, as in the case of herbicides, replanting may be recommended to take place only between the treated bands.

(ii) *Rotational crops (including cover crops).* If detrimental effects are observed, results of studies evaluating severity and duration of effects on the injured rotational crops must be submitted. To determine the duration of phytotoxic effects, susceptible rotational crops must be planted at varying time intervals after pesticide application. Such studies may be combined with field studies designed to evaluate soil residues. [See § 163.62–11(b) of Subpart D.]

Tier 1 Testing

§ 163.122–1 Seed germination/seedling emergence and vegetative vigor (Tier 1).

(a) *When required.* Data on the toxic effects of a pesticide on seed germination or seedling emergence and vegetative vigor are required to support the registration of each formulated product intended for outdoor pesticide application, and each manufacturing-use product which legally could be used to make such formulated products.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3, the seed germination or seedling emergence and vegetative vigor studies shall meet the following standards:

(1) *Test substance.* The technical or purer grade of the active ingredient shall be tested.

(2) *Species.* The following species shall be tested:

Lycopersicon esculentum (tomato)
Cucumis sativus (cucumber)
Lactuca sativa (lettuce)
Glycine max (soybean) (inoculated with *Rhizobium japonicum* for vegetative vigor study)
Brassica oleracea (cabbage)
Avena sativa (oat)
Lolium perenne (perennial ryegrass)
Allium cepa (common onion)
Daucus carota (carrot)
Zea mays (corn)

(3) *Dose levels.* One concentration level equal to 3 times the maximum label rate shall be tested. The phrase "3 times the maximum label rate" means 3 times the amount of active ingredient in the recommended quantity of carrier such as water to be used per land area. For purposes of calculating the dose level in the seed germination/seedling emergence study, 1 pound of active ingredient per acre is considered to be equal to 10 ppmw in the solution which is applied to seeds.

(4) *Number of plants.* At least 3 replicates, each with 5 plants, shall be tested per dose level for the vegetative vigor tests. At least 3 replicates, each with 10 seeds, shall be tested per dose level for the seed germination study.

(5) *Site.* The seed germination/seedling emergence studies shall be conducted under controlled conditions in growth chambers. The vegetative vigor test may be performed in a growth chamber, greenhouse, or in small field plots.

(6) *Duration.* (i) Seed germination/seedling emergence shall be observed weekly, or more frequently, for at least 2 weeks. See the Appendix, Item (1) for details of this study.

(ii) The effect on vegetative vigor shall be observed weekly, or more frequently, for at least 2 weeks. If abnormal symptoms occur, the observations shall be continued until the plant dies or fully recovers. See the Appendix, Item (2) for details of this study.

(7) *Protocols.* The protocols for these tests outlining the acceptable environmental conditions, procedures, and some pertinent references are found in the Appendix, Items (1) and (2).

(c) *Reporting.* In addition to the information required in § 163.120–4(b), the test report shall include the following information.

(i) The actual number of seeds tested and the number germinated or emerged per dosage level for each replicate;

(ii) Descriptions of the appearance and the growth and development (height and weight) of the seeds and emergent plants, indicating any abnormalities and expressions of phytotoxicity; and

(iii) Tabulation of the results indicating the percentage effect level for each species as compared to untreated control plants.

(d) *Tier progression.* (i) If a detrimental effect or response on plant growth and development for any plant species for the 3X concentration is 10 percent or greater with respect to the control, testing at tier 2 is required. See § 163.123–1.

(ii) If less than a 10 percent detrimental effect or response is noted, no additional testing at higher tiers is ordinarily required. The Agency, after review of the data, may require certain additional tests to determine a more definite nondiscernible effect level.

§ 163.122–2. Growth and reproduction of aquatic plants (Tier 1).

(a) *When required.* Data on the toxic effects of a pesticide on growth and reproduction of aquatic plants are required to support the registration of each formulated product intended for outdoor pesticide application, and each manufacturing-use product which legally could be used to make such formulated products.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3, studies of the growth and reproduction of aquatic plants shall meet the following standards:

(1) *Test substance.* The technical or purer grade of the active ingredient shall be tested.

(2) *Species.* The following species shall be tested:

Lemna gibba (duckweed)

Selenastrum capricornutum (freshwater green alga)

Skeletonema costatum (marine diatom)

A freshwater diatom (unspecified species)

Anabaena flos-aquae (blue-green alga)

(3) *Dose levels.* The quantity of test substance to be tested shall be equivalent to a 3-fold (or 3X) concentration of the maximum label rate as though it were directly applied to the surface of a 15-cm or 6-inch water column. The phrase "3-fold concentration of the maximum label rate" means 3 times the formulation or active ingredient content in the recommended quantity of carrier such as water to be used per aquatic area or volume.

(4) *Number of plants.* At least 3 replicates, each with 5 vascular aquatic plants (*Lemna gibba*) (stage: 3 fronds per plant) shall be tested per dose level. The recommended quantities of algal plant material to be used are provided in the recommended references of the protocols provided in the Appendix, Items (4), (5), (6), and (7).

(5) *Site.* All studies provided for in this section shall be conducted under controlled conditions in growth chambers.

(6) *Duration.* (i) *Lemna* studies shall be conducted for at least 14 days with daily observations. If abnormal symptoms occur, the observations shall be continued until the plants recover or die.

(ii) Algal studies shall be conducted for at least 5 days with daily observations. If abnormal symptoms occur, the observations shall be continued until the plants recover or die.

(7) *Protocols.* The protocols for these tests outlining the acceptable environmental conditions and procedures and some pertinent references are found in the Appendix, Items (3) through (7).

(c) *Reporting.* In addition to the information required by § 163.120–4(b)(1) through (5), and (8), of this subpart, the test report shall include the following:

(1) *Lemna.* (i) The percent of flowering or its inhibition; and

(ii) The increase in growth expressed as the number of original plants and fronds and the additional plants and fronds produced;

(2) Algae. (i) The gram dry weight;

(ii) The cell count per ml; and

(3) Tabulation of the results indicating the percentage effect level versus time as compared to the control.

(d) *Tier progression.* (1) If a detrimental effect or response on plant growth and development for any plant species for the 3X concentration is 50 percent or greater with respect to the controls, testing at tier 2 is required. See § 163.123–2.

(2) If less than a 50 percent detrimental effect or response is noted, no additional testing at higher rates is required. The Agency, after review of the data, may require certain additional tests to determine a more definite nondiscernible effect level.

§ 163.122–3 Mutagenicity effects (Tier 1).

(a) *When required.* (1) Data on the mutagenic effect of a pesticide on plants are required to support the registration of each formulated product which:

(i) Is intended for outdoor application, and

(ii) Contains any ingredient producing a mutagenic effect in tests conducted according to §§ 163.84–1 through –4 of Subpart F.

(2) These data are also required to support the registration of each manufacturing-use product which legally could be used to make such formulated products.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3, the mutagenic effects studies shall meet the following test standards:

(1) *Test substance.* The technical or purer grade of the active ingredient shall be tested.

(2) *Species.* The following species shall be tested: *Tradescantia reflexa.*

(3) *Dose levels.* (i) At least 5 dosage levels shall be tested. The doses shall include a toxic concentration, a subtoxic concentration, and a concentration that does not induce any toxicity.

(ii) The dosage levels shall be of geometric progression of no more than 2-fold. For example, the test concentration series may be: 1, 2, 4, 8, 16, and 32 ppm (a 2-fold progression).

(4) *Number of plants.* At least 3 replicates, each with 5 plants, shall be tested per dose level for the mutagenic effects test.

(5) *Duration.* The mutagenic effects test shall extend for 10 days with daily observations.

(6) *Site.* This study shall be conducted in the laboratory under controlled conditions.

(7) *Protocols.* The protocols for these tests outlining the acceptable environmental conditions and procedures and some pertinent references are found in the Appendix, Item (8).

(c) *Reporting.* In addition to the information required by § 163.120–4(b)(1) through (4), the test report shall include the following information:

(i) The number of pink cells (mutagenically-affected) in the strings of otherwise blue (normal) staminal hair cells; and

(ii) Tabulation of the results indicating the percentage effect level versus time as compared to the control. The test report shall include the determination of the 10, 50, and 90% detrimental effect levels, and the 95% confidence limits for each.

(d) *Tier progression.* (i) If any of the dosage levels employed in this test produces a mutagenic incidence exceeding the EC10 for the mutagenicity test, testing at the tier 3 level is required. See § 163.124–3 which expands the number of plants to be tested.

(ii) If less than 10 percent effect is noted in this test, no additional testing at a higher tier is ordinarily required. The Agency, after review of the data, may require certain additional tests to determine a more definite non-discernible effect level.

Tier 2 Testing

§ 163.123–1 Seed germination/seedling emergence and vegetative vigor (Tier 2).

(a) *When required.* Data on the toxicity effects of a pesticide on seed germination or seedling emergence and vegetative vigor are required at the tier 2 level to support the registration of each formulated product intended for outdoor application, and each manufacturing-use product which legally could be used to make such formulated products, if the results of the tier 1 tests required by § 163.121–1 have indicated an adverse effect on growth and development greater than 10 percent on plant species.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3, the test standards for this section shall be the same as those contained in the tier 1 studies [§ 163.122–1(b)] with the following modifications:

(1) At least 5 dosage levels shall be tested;

(2) The doses shall include a toxic, subtoxic, and a concentration that does not induce any toxicity;

(3) The highest dosage levels shall be less than the 3-fold dose level tested in § 163.122–1(b)(3); and

(4) The dosage levels shall be of geometric progressions of no more than 2-fold. For example, the test concentration series may be: 0.1, 0.2, 0.4, 0.8, and 1.6 kg/ha (a 2-fold progression).

(c) *Reporting.* In addition to those items required in § 163.122–1(c), the test shall include determination of the 10, 50, and 90 percent detrimental effect levels and the 95 percent confidence limits for each.

(d) *Tier progression.* (1) Testing at the tier 3 level is required if the calculated expected exposure concentration (the maximum recommended rate or concentration) is greater than the EC10 for any terrestrial plant species tested. (Tier 3 testing increases the number of species to be tested.) See § 163.124–1.

(2) Testing at the tier 4 level may be required if, in addition to the criterion described in paragraph (d)(1) of this section:

(i) The geographical use is potentially widespread (two or more regions); testing according to § 163.125–1 is then required. The area of testing shall be expanded to include all of those geographical regions defined in § 163.125–1(b) where the product is expected to be used. This requirement and that of testing under § 163.124–1 may be met by performing combined testing.

(ii) The applications include varying periods or seasons of application; testing according to § 163.125–2 is then required. The more susceptible plant life stages that correspond to those encountered during field application shall be evaluated.

(iii) The material is readily sorbed on soil solids; testing according to § 163.125–4 is then required, to assess the effect of the pesticide on the growth of aquatic plants. Employ sediment, particle, and solution concentrations that may be expected under natural conditions.

(3) If there is a marked reduction in growth of soybean (§ 163.123–1) and *Anabaena* (§ 163.123–2), as compared to other species tested in tier 2, then testing must be undertaken to assess the effect of the pesticide on the ability of certain organisms to fix nitrogen, as described in § 163.125–3. [See also § 163.62–8(f)(3) of Subpart D for related nitrogen fixation studies.]

§ 163.123–2 Growth and reproduction of aquatic plants (Tier 2).

(a) *When required.* Data on the toxic effects of a pesticide on growth and reproduction of aquatic plants are required to support the registration of each formulated product intended for outdoor pesticide application, and each manufacturing-use product which legally could be used to make such formulated products, if the results of the tier 1 tests required by § 163.122–2 have indicated an adverse effect on growth and reproduction of any aquatic plant greater than 50 percent.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3, the test standards for this section shall be the same as those contained in the tier 1 studies [§ 163.122–2(b)] with the following modifications:

(1) At least 5 dosage levels shall be tested;

(2) The dose levels shall include a toxic, subtoxic, and a concentration that does not induce any toxicity;

(3) The highest dosage level shall be less than the 3-fold concentration tested in § 163.122–2(b)(3); and

(4) The dosage levels shall be of geometric progression of no more than 2-fold. For example, the test concentration series may be: 0.1, 0.2, 0.4, 0.8, and 1.6 kg/ha/15 cm (a 2-fold progression).

(c) *Reporting.* In addition to the information required by § 163.122–2(c), the test report shall include the determination of the 10, 50, and 90 percent detrimental effect levels and the 95 percent confidence limits for each.

(d) Tier progression. (1) Testing at the tier 3 level is required if:

(i) The calculated expected exposure quantity [the maximum recommended quantity where 1 kg/ha (0.892 lb/A) = 0.655 ppm in 15 cm (6") of water] is greater than the EC50 for any one aquatic plant species tested; and

(ii) The pesticide is expected to be applied to a fresh water, estuarine, or marine aquatic system by either direct application or direct discharge of treated water, or the pesticide is to be used within a forest system. (A forest system is considered equivalent to an aquatic system, since it ordinarily contains brooks, streams, and rivers. See § 163.62–6 (b), (c), and (d) of Subpart D for full explanation of pesticide aquatic use patterns.) See § 163.124–2 (tier 3) where the number of species to be tested has been expanded. In addition to this criterion:

(2) Testing at the tier 4 level may be required if, in addition to the criterion described in paragraph (d)(1) of this section:

(i) The geographical use is potentially widespread (two or more regions); then testing according to § 163.125–1 is required. The area of testing shall be expanded to include all of those geographical regions defined in § 163.125–1(b) where the product is expected to be used. This requirement and those under § 163.124–2 may be met by performing combined testing.

(ii) The applications include varying periods or seasons of application; then testing according to § 163.125–2 is required. Potentially susceptible plant life stages which correspond to those encountered during field application shall be evaluated.

(iii) The material is readily sorbed on soil solids; then testing according to § 163.125–4 is required, to assess the effect of the pesticide on the growth of aquatic plants. Employ sediment, particle, and solution concentrations that may be found under natural conditions.

(3) If there is a marked reduction in growth of soybean (§ 163.123–1) and *Anabaena* (§ 163.123–2) as compared to other species tested in tier 2, then testing must be undertaken to assess the effect of the pesticide on the ability of certain organisms to fix nitrogen, as described in § 163.125–3. [See also § 163.62–8(f)(3) of Subpart D for related nitrogen fixation studies.]

Tier 3 Testing

§ 163.124–1 Terrestrial field testing (Tier 3).

(a) *When required.* (1) Data on the toxic effects of the active ingredient (in the form of a typical formulation) on seed germination, vegetative vigor, and reporduction patential of an expanded number of terrestrial plants are required to support the registration of each

formulated end-use product intended for outdoor application, when the calculated exposure quantity (the maximum recommended quantity) is equal to or greater than the EC10 for any terrestrial plant species as found in the tier 2 tests (§ 163.123–1).

(2) Data as indicated in paragraph (a)(1) of this section are also required to support the registration of each manufacturing-use product which legally could be used to make formulated end-use products that are intended for outdoor application.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3, the test standards for this section shall be the same as those contained in § 163.122–1(b) of this subpart, with the following modifications:

(1) *Test substance.* The test substance shall be a typical formulated end-use product representative of a major formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) and containing the active ingredient of the applicant's product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product which legally could be used to make an end-use product with outdoor uses, the test substance shall be a product representative of the major formulation category which includes that end-use product. (If the end-use products with outdoor uses that could be made from the manufacturing-use product belong to two or more major formulation categories, a separate study must be performed for each such category.)

(2) *Dose levels.* The dose levels tested shall be the same as those employed in the tier 2 test [§ 163.123–1(b)(1)].

(3) *Species.* (i) Representatives of the following plant groups are to be tested, subject to the limitations of paragraph (iii) below:

(A) *Dicotyledonae* (dicots), representatives of three families;

(B) *Monocotyledonae* (monocots), representatives of three families;

(C) Vascular *Cryptogamae* (ferns and allies), representatives of two families;

(D) *Bryophyta* (mosses and liverworts), one representative; and

(E) *Gymnospermae* (conifers), one representative.

(ii) Plant species used for testing tiers 1 and 2 cannot be used to satsify the test requirements of this section.

(iii) If any of the plant groups are not likely to be exposed to the pesticide under normal conditions of use, testing of such groups is not required. Justification for elimination of a test species or group must be included in the test report.

(4) *Test conditions.* (i) Plants may be grown in either natural soil or similar substrate maintaining approximately the same edaphic conditions as found in the natural habitat. These conditions must be reported.

(ii) The light quality and quantity must be similar to that in the natural environment.

(iii) Other test conditions such as relative humidity and temperature must be similar to those of the natural habitat of the plants.

(c) *Reporting.* In addition to the information required in §§ 163.120–4 and 163.122–1(c) of this subpart, the test report shall include the determination of the 10, 50, and 90 percent detrimental effect levels and the 95 percent confidence limits for each.

§ 163.124–2 Aquatic field testing (Tier 3).

(a) *When required.* (1) Data on the toxic effects of the active ingredient (in the form of a typical formulation) on growth and reproduction of an expanded number of aquatic plants are required to support the registration of each formulated end-use product intended for outdoor pesticide application, when:

(i) The calculated expected exposure is equal to or greater than the EC10 for any one aquatic plant species tested in tier 2 tests (§ 163.123–2); and

(ii) The pesticide is expected to be applied to a fresh water, estuarine, or marine aquatic system by either direct application or direct discharge of treated water, or the pesticide is to be used within a forest system. [See § 163.62–6(b), and (d) of Subpart D for descriptions of these aquatic uses.]

(2) Data as indicated in paragraph (a)(1) of this section are also requred to support the registration of each manufacturing-use product which legally could be used to make such a formulated product.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3 of this subpart, the test standards for this section shall be the same as those in § 163.122–2(b), with the following modifications:

(1) *Test substance.* The test substance shall be a typical formulated end-use product representative of a major formulation category (e.g., emulsifiable concentrate, granular product, wettable powder) and containing the active ingredient of the applicant's product.

(i) If the applicant's product is a manufacturing-use product which legally could be used to make an end-use product with outdoor uses, the test substance shall be a product representative of the major formulation category which includes that end-use product. (If the end-use products with outdoor uses that could be made from the manufacturing-use product belong to two or more major formulation categories, a separate study must be performed for each such category.)

(2) *Dose levels.* The dose levels tested shall be the same as those specified in the tier 2 aquatic test standards [§ 163.123–2(b)(1)].

(3) *Species.* (i) Aquatic plant representatives of the following plant groups are to be tested:

(A) *Dicotyledonae* (dicots), one representative;

(B) *Monocotyledonae* (monocots), representatives of three families;

(C) Vascular *Cryptogamae* (ferns and allies), representatives of three families;

(D) Algae (including *Cyanophyta*), a representative of each Division; and

(E) *Bryophyta*, one representative.

(ii) Plant species used for testing tiers 1 and 2 cannot be used to satisfy the test requirements of this section.

(4) *Environmental conditions.* (i) Plants may be grown in either native soil, water, or other substrate of similar nature to that of the indigenous area or under other conditions similar to the natural habitat. The edaphic conditions must be reported.

(ii) Reduction of light intensity by natural or constructed light shade may be necessary to simulate the reduced light intensities found with certain plant communities such as deeply submerged sites or shaded waters.

(iii) Other natural conditions must also be maintained where plants are removed from their natural habitat. Soil, water, and air temperatures should approximate those of the natural habitat. For estuarine and marine habitats, the following conditions should, to the extent possible, simulate the natural environment: tidal action, wave action, water turbidity, flow rates, depth, salinity, and degree of exposure.

(iv) Tests must be performed either in enclosed, controlled areas of a lake, pond, or swamp, or in large water cultures such as aquaria or plastic wash tubs. Tests are not to be performed in dynamic or flowing water where the release of the chemical cannot be contained or its escape prevented.

(v) The performance of the field studies shall be conducted using acceptable protocols as may be found in the following recommended reference: Truelove, B., 1977, Research Methods in Weed Science, Southern Weed Science Society, Auburn Printing Inc., Auburn,

AL; *or* by a protocol with prior approval of the Agency.

(c) *Reporting.* In addition to the information required by §§ 163.120–4 and 163.122–2(c) of this subpart, the test report shall include the determination of the 10, 50, and 90 percent detrimental effect levels and the 95 percent confidence limits for each.

§ 163.124–3 Mutagenicity tests (Tier 3).

(a) *When required.* Data on the mutagenic effects of a pesticide on an expanded number of plant species are required to support the registration of each formulated product intended for outdoor pesticide application, and each manufacturing-use product which legally could be used to make such formulated products, when the tier 1 test (§ 163.122–3) indicates incidence of mutagenic effects on plants exceeding 10 percent.

(b) *Test standards.* In addition to the general test standards set forth in § 163.120–3 of this subpart, the test standards for this section shall be the same as those in § 163.122–3(b), with the following modification as to species:

Glycine max (soybean)
Vicia faba (broad bean)
Allium cepa (common onion)
Zea mays (corn).

(c) *Reporting.* The reporting requirements shall be the same as those specified for the tier 1 mutagenicity tests [§ 163.122–3(c)] of this subpart.

Tier 4 Testing

§ 163.125–1 Widespread geographical uses (Tier 4).

(a) *When required.* Data on the toxic effects of the active ingredient (in the form of a typical formulation) on growth and development of plants in those regions where the product is expected to be used are required to support the registration of each formulated end-use product intended for outdoor terrestrial or aquatic pesticide applications, and each manufacturing-use product which legally could be use to make such formulated products, when:

(1) The calculated expected exposure quantity is greater than the EC10 for plants as determined in the tier 2 tests (§ 163.123–1 or 163.123–2); and

(2) The product use is expected to be widespread (two or more regions).

(b) *Test standards.* In addition to the test general standards set forth in § 163.120–3, the test standards for this section shall be the same as those in § 163.124–1(b) (for terrestrial plants) and § 163.124–2(b) (for aquatic plants) depending on target area, with the following modifications:

(1) The following United States geographical areas must be considered: northeastern temperate deciduous, southeastern temperate deciduous, northern grassland (prairie), southern grassland (prairie), northwestern (and Alaskan) conifer forest and high desert, southwestern chapparal Mediterranean and low desert, and Hawaiian and Caribbean tropical forest. Where important species diversity and phsiographic differences occur within a region of intended application, the regional testing may be inadequate, and testing at a more specific region or biome level will be required.

(2) Those areas where tests are to be conducted are those where the product would be expected to be used.

(c) *Reporting.* The test reports shall provide the information required in §§ 163.124–1(c) and 163.124–2(c).

§ 163.125–2 Widespread seasonal use (Tier 4).

(a) *When required.* Data on the toxic effects of the active ingredient (in the form of a typical formulation) on plant growth and development under different environmental conditions are required to support the registration of each formulated end-use product intended for outdoor terrestrial and aquatic pesticide application, and each manufacturing-use product which legally could be used to make such a formulated product, when:

(1) The calculated expected exposure quantity is greater than the EC10 for plants as determined in the tier 2 tests (§§ 163.123–1 and 163.123–2); and

(2) The applications are expected to include varying periods or seasons of application.

(b) *Test standards.* The test standards for this section shall be identical to those in § 163.124–1(b) for terrestrial plants and in § 163.124–2(b) for aquatic plants with the following modification: the test substance is to be applied over the period of time or season according to the proposed label instructions.

(c) *Reporting.* The test reports shall provide the information required in §§ 163.124–1(c) and 163.124–2(c). The report shall contain an evaluation of the pesticide effects over a period of time and with the repeated applications.

§ 163.125–3 Nitrogen fixation potential (Tier 4).

(a) *When required.* Data on the effect of a pesticide on nitrogen fixation potential are required to support the registration of each formulated product intended for outdoor pesticide use, and each manufacturing-use product which legally could be used to make such formulated products, if there is a marked reduction in growth of soybean (§ 163.123–1) and *Anabaena* (§ 163.123–2), as compared to other species tested in tier 2. [See also § 163.62–8(f)(3) of Subpart D for related nitrogen fixation data requirements.]

(b) *Test standards.* The test standards for this section shall be identical to those for tier 1 and 2 studies [§§ 163.122–1(b), 163.123–1(b), 163.122–2(b), and 163.123–2(b)] with the following modifications:

(1) *Species.* The following species shall be used:

Trifolium repens (white clover)
Glycine max (soybean)
Azolla spp. (waterfern)
Azotobacter vinelandii
Anabaena flos-aquae

(2) *Protocol.* (i) The acetylene reduction method is to be used to measure the nitrogen fixation potential.

(ii) The individual protocols for the legumes, *Azolla, Azotobacter,* and *Anabaena* are provided in the Appendix, Item (9).

(c) *Reporting.* The test report shall include the test data and the determination of the 10, 50, and 90 percent (I10, I50, and I90) detrimental effect levels on nitrogen fixation potential and the associated 95 percent confidence limits for each species. The data shall be expressed as moles of ethylene produced per gram of fresh or dry weight per time period.

§ 163.125–4 Readily sorbed materials (Tier 4).

(a) *When required.* Data on the effects of sorbed pesticides on aquatic plant life are required to support the registration of each formulated product intended for outdoor pesticide application, and each manufacturing-use product which legally could be used to make such formulated products, when the pesticide is readily sorbed to materials such as soils and organic matter that are likely to be carried into aquatic systems. Extent of sorption is determined in the absorption/desorption studies of § 163.62–9(d) of Subpart D.

(b) *Test standards.* In addition to those test standards required in § 163.123–2(b), the following standards shall be met:

(1) *Protocol.* An aquatic ecosystem approach may be used to assess the effect of sorbed pesticide materials. The pesticide at the appropriate concentrations should be mixed with the substrate and allowed to stand for 24 hours before filling the tanks or trays. Filling must be performed in a manner as to minimize substrate turbulence. The concentration in the soil and the filtered water should then be determined. Where turbulent conditions are desired in order to stimualte mixing or turbulent conditions in aquatic systems, the turbulence should be uniform and the

amount of suspended particulate matter and its pesticide content determined.

(c) *Reporting.* In addition to the information required by § 163.123–2(c), the following data are required:

(1) The concentration of the pesticide in the sediment and water after equilibrium is attained;

(2) The concentration of the pesticide in the suspended particles during the turbulent conditions; and

(3) The concentration of the pesticide within the plants at the termination of the study.

Special Testing

§ 163.126–1 Spray drift: General requirements.

(a) *When required.* (1) Data on the toxic or hazard effects and extent of the potential drift of a pesticide are required to support the registration of each formulated product expected to be applied by aerial application spray methods (fixed-wing and helicopter), by air carrier (mist blower) method, or by other ground spray application methods. Such ground spray application methods may include, but are not limited to, overhead sprinkler irrigation devices, roadside and railroad right-of-way sprayers, and tractor-mounted booms.

(2) (i) The requirement to submit test data established by § 163.126–2 (Plant activity spectrum) may be satisfied by the submittal of published or unpublished information on plant spectrum studies such as are required in §§ 163.123–1 and –2 of this subpart.

(ii) The requirement to submit test data established by §§ 163.126–3 (Droplet size spectrum) and 163.126–4 (Field evaluation) may be satisfied by the submittal of published or unpublished information regarding spray drift patterns that would be expected to be similar to those for the formulated product when used according to commonly-recognized practice. Such submitted information must be accompanied by a statement of reasons why the registration applicant considers that the characteristics of his product are similar or identical to those of the desginated formulation described in the published information.

(3) If the applicant chooses not to furnish published and unpublished information as provided by paragraphs (a)(2)(i) and (ii) of this section, he may use the requirements of this section as guidance for studies he may wish to conduct to support his product if it may, for example, have superior or different characteristics regarding spray drift patterns and resultant phototoxicity than published or unpublished test data would demonstrate.

(b) *Data requirements.* The drift hazard of a peticide applied either by aircraft or ground equipment shall be determined in three stages. The initial stage (see § 163.126–2) involves the determination of possible pesticidal effects on plant growth activity that may be produced through direct foliar spraying and through exposure to pesticide-contaminated (spiked) soil. The second stage (see § 163.126–3) involves determination of droplet size spectrums to indicate which of the conventionally-used nozzle types, orifice sizes and cores, operating conditions, adjuvants, formulations, and discharge orientations would produce the greatest probable drift potential (for example, greatest volume of droplets less than 100 microns in diameter). The final stage (see § 163.126–4) involves field studies using commercial equipment to determine the extent of spray drift (concentration vs. distance) from swath displacement under "worst case" equipment and environmental (e.g., inversion) conditions. Data required by §§ 163.126–3 and –4 (Droplet size spectrum, and Spray drift evaluation) will be submitted only when the maximum label dosage produces a 10 percent detrimental effect or greater on the most sensitive plant(s) as tested in accordance with § 163.126–2. Determination of the spray droplet size spectrum (§ 163.126–3) may be performed before undertaking or during the aerial spray drift field study evaluation using commercial equipment (§ 163.126–4).

(c) *Additional data.* Submission of data from §§ 163.126–2, –3, and –4 will allow evaluation of the effect of swath displacement to nontarget plants. If, upon submission of data on activity levels as described in § 163.126–2, there are no effects of the pesticide on nontarget plants, additional data on spray drift may not be necessary with respect to nontarget plant hazard. If the addition of new crops, new uses, or new methods of application to the labeling of a registered product is planned by the registrant, additional testing as described in §§ 163.126–3 and –4 of this subpart may be required.

§ 163.126–2 Spray drift: Plant activity spectrum studies.

(a) *When required.* Data on the toxic effects of a pesticide on vegetative vigor are required to support the registration of each formulated product expected to be applied by aerial application spray methods, by air carrier (mist blower) methods, or by other ground spray application methods. Data may be supplied through either of the following alternative means:

(1) Where the effects on vegetative vigor have been determined according to the tier 1 and 2 terrestrial plant tests (§§ 163.122–1 and 163.123–1) using the specific formulation [or formulations similar to that of the proposed formulated product (e.g., a 4EC can be substituted for a 6EC, etc.)], data employing the formulation(s) may be submitted; or

(2) Data from screening tests performed by the registrant using the specific formulation (or formulations similar to that of the proposed formulated product) may be submitted in place of the studies as noted in this section. The Agency will reserve the right to require testing as provided by this section if the submitted data do not prove to be adequate to answer the question(s) concerning a pesticide's phytotoxic nature.

(b) *Test standards.* In addition to those test standards set forth in § 163.120–3, the following test standards shall be substituted for the appropriate portions of the tier 2 vegetative vigor test [§ 163.122–1(b)]:

(1) *Test substance.* The formulated product shall be tested. Similar formulations of a particular type may be substituted in the study (such as a 4EC for a 6EC formulation).

(2) *Plant species.* (1) Plant species evaluated must include those species or representatives of the families listed in § 163.121–1(b)(2) and any other plant species or family representatives of known high susceptibility to the product. Any additional plant species selected must be representative of those species found in the geographical area(s) of the United States where the product is expected to be used.

(ii) Culture of bioassay plants must be described both prior to and subsequent to exposure. Plants may be greenhouse-, growth chamber-, or field-grown.

(iii) Stages of nontarget plant growth at application must encompass those stages encountered under actual use situations.

(c) *Reporting.* The information required in §§ 163.120–4(b) and 163.123–1(c) shall be reported. The applicant must report product activity on sensitive nontarget plants that may be affected through direct foliar contact by spray, and through exposure to pesticide-contaminated soils. The latter exposure represents those situations where plants are grown in soil contaminated with pesticides that have drifted or have been irrigated with water that has been contaminated with the pesticide.

(d) *Test progression.* If the product is to be applied by aerial, air carrier (mist blower), or other ground spray application methods, and the

detrimental effect is greater than 10% for any of the species as compared to the untreated controls, then the droplet size distribution shall be determined (see § 163.126–3) and the product shall be evaluated according to § 163.126–4, employing commercial application equipment.

§ 163.126–3 Spray drift: Droplet size spectrum studies.

(a) *When required.* (1) Data on the droplet size spectrum are required to support the registration of each formulated product expected to be applied by aerial, air carrier mist blower, and by other ground spray application equipment when the detrimental effect level of those desirable plants anticipated to be present exceeds 10 percent as determined by plant activity spectrum tests of § 163.126–2.

(2) In lieu of the wind tunnel study required by this section, droplet size distribution may be determined under field conditions during the spray drift evaluation test as provided in § 163.126–4.

(b) *Test standards.* In addition to those test standards set forth in § 163.120–3, the following standards shall be met:

(1) *Equipment.* The label-recommended or commonly-used nozzles and associated parts, nozzle pressures, and nozzle discharge orientation that would produce droplets that would be most conducive to spray drift shall be tested.

(2) *Meteorological conditions.* (i) For wind tunnel studies, the following conditions shall be tested:

(A) The product shall be tested at various temperature levels from 10 to 30°C.

(B) The air flow (velocity) in the wind tunnel will be adjusted to relate to the type of equipment used [e.g., 130 to 225 kmph (80 to 140 mph) for fixed-winged aircraft and air carriers, 65 to 110 kmph (40 to 70 mph) for helicopter, and 5 to 40 kmph (3 to 25 mph) for ground applications other than air carriers (mist blowers)].

(ii) For field determination studies, the meteorological conditions shall be those most conducive to spray drift (high temperature and low relative humidity).

(3) *Collection devices.* The collection devices shall be either remote sensing laser-powered particle spectrometers, collection cards (flat horizontal or vertical surfaces), or other devices by which droplet size distribution can be determined.

(c) *Reporting.* In addition to those reporting requirements set forth in § 163.120–4, the following data are required:

(1) The nozzle type, orifice size, and core identification;

(2) The nozzle pressure and flow rate;

(3) The nozzle discharge orientation to the airstream;

(4) Air velocity past the nozzle;

(5) Descriptions of techniques and size determination devices;

(6) Particle size distribution vs. cumulative percent volume and droplet number (an attempt should be made to determine the droplet size distribution as the droplets leave the nozzle);

(7) Product formulation, dilution, mixtures, adjuvants, and their physical properties (surface tension, viscosity, density, vapor pressure, etc); and

(8) Air temperature and relative humidity (not required for wind tunnel studies where droplets at the nozzle are measured).

§ 163.126–4 Spray drift: Field evaluation.

(a) *When requried.* (1) Data on the extent of the potential drift of a pesticide are required to support the registration of each formulated product expected to be applied by aerial, air carrier, (mist blower), or other ground spray application methods when the detrimental effect level of those desirable plants anticipated to be present exceeds 10 percent as determined by the plant activity spectrum tests of § 163.126–2.

(2) Experimental use permits will usually be required for conduct of these field evaluations.

(b) *Test standards.* In addition to the test standards set forth in § 163.120–3, the following test standards shall be met:

(1) *Test substance*—(i) *Products with single active ingredients.* The formulated product shall be tested. Use of dyes or other indicators with the pesticide are acceptable only if these materials do not interfere with chemical analysis or bioresponse, do not alter chemical or physical properties of the diluted spray, do deposit in direct proportion to concentrations of active ingredients, and do remain stable until analysis.

(ii) *Tank mixes and package mixes.* Drift data requirements for each package mix, and for each tank mix allowed in labeling are identical to those requirements for any single pesticide applied alone; that is, the plant activity spectrum, droplet spectrum, and swath displacement must be evaluated. Alternatively, the swath displacement data for the mixture may not be required if the mixture activity spectrum, droplet size spectrum, and physical data are sufficiently similar to such data characterizing the mixture's most phytotoxic component for which acceptable swath displacement data have been submitted.

(2) *Dosage levels.* Maximum label-recommended pesticide dosages must be evaluated in all spray drift field evaluations.

(3) *Location.* The evaluation should preferably be performed in a field having foliage or crop cover conditions similar to actual use conditions. The site should be typical in geography, topography, and meterology of those sites within intended use patterns and seasonally similar in relation to the test plants. Open fields (not runways) will be acceptable only for evaluation of pesticides to be applied directly to soil or just emerging plants. If it is felt that barren fields should be used to test where vegetation would exist according to the label recommendations, the justification must be submitted to indicate the reason for this test modification.

(4) *Collection surfaces.* Collection surfaces for fallout sampling must be located within the target area and at least one swath width upwind and downwind at known distances outside the target area to a minimum of 1000 feet for aerial and air carrier application and 500 feet for other ground applications. The surfaces may be either inanimate or biological. Collection surfaces must be located in a straight line parallel to a stable wind direction and as close as possible to the perpendicular of the direction of travel for the application equipment. At each collection station, collection surfaces must be located at the soil surface or, if vegetation is present, at a level which corresponds to the height of the surrounding canopy. The downwind and upwind line of collection stations must extend at least to the point where quantities of pesticide collected are less than the no-effect level of the most sensitive plant species evaluated in § 163.126–2(b). Air samplers, such as a high volume type (20–50 ft^3/min), must be placed at a 2- or 3-meter height at least three downwind collection stations (for example, at 200, 600, and 1000 feet downwind) to sample airborne particles of the chemical. While lateral distances between collection stations are left to the discretion of the applicant, it is important that a sufficient number of collecton stations be established to present a definitive uninterrupted picture of deposits across the treated swath as well as outside the target area. Location of collection stations is particularly critical in areas where deposition rates are expected to

change rapidly over a small lateral distance.

(5) *Protocols.* (i) Spray drift evaluation data from one or more applications to a single swath line will be acceptable. When plant bioassays are used in the swath displacement test, only single applications over the swath test area may be performed. The length of the treated swath must be such that a line of continuation of the spray cloud would pass above the most distant downwind collection devices, taking into account the probability that wind direction may not coincide perfectly with the line of collection points. Normally the spray line should equal the sampling line length.

(ii) At least one study must be conducted using equipment, equipment adjustment and operation, procedures, and conditions most conducive to drift as would be allowed in labeling, and as determined in part from droplet spectrum tests. As an example, evaluate the spray drift using the following parameters that would be permitted in normal application practices: maximum recommended nozzle height, nozzle pressure, crosswind velocity, temperature gradient at 3 and 10 m, ground speed of the equipment, pesticide dosage, and airstream shear force (nozzle discharge orientation to air stream); minimum recommended nozzle orifice diameter, spray volume and relative humidity; and using cores any other devices to increase droplet dispersion or produce sheets, fans, or cones.

(c) *Reporting.* In addition to data reporting information required by § 163.120–4, the following information must be submitted:

(1) A diagram of the plot indicating north, swath width, and orientation, and location and spacing of the collection stations;

(2) Temperature at two levels, wind velocity and direction, variations in velocity and direction during the application, relative humidity, atmospheric pressure, and air stability. The latter is expressed as Barad's stability ratio:

$$\frac{T_{10}\text{ meters} - T_3\text{ meters}}{(\text{ave. wind vel.})^2} \times 10^5$$

Temperture and wind velocity shall be determined at the 3 and 10 meter height for aerial and air carrier applications and for all other ground applications;

(3) Dosage of active ingredients or acid equivalent per hectare (or acre). The concentration of the final diluted spray mixture (in the spray tank) must be sampled, chemically analyzed, and reported;

(4) Physical property data, including droplet size, viscosity, density, vapor pressure, visco-elasticity, and surface tension;

(5) Spray volume (liters per hectare or gallons per acre) and carrier(s). The maximum and minimum carrier volumes recommended on the label must be reported;

(6) Adjuvant identification and dilution used;

(7) The ground speed of application equipment, the number of swaths sprayed during exposure of collecting surfaces, and swath width;

(8) A description of the spray equipment, including nozzle type, orifice, size, core, nozzle pressure, nozzle spacing and arrangement, and nozzle discharge orientation. Nozzle discharge orientation shall be designated in degrees related to the direction of travel of the spray equipment. Zero degrees indicates a horizontal nozzle discharge pointing rearward (opposite to the equipment direction of travel), 90° indicates a vertical nozzle discharge pointing downward, and 180° indicates a horizontal nozzle discharge pointing forward (with the equipment direction of travel);

(9) The minimum and maximum nozzle-to-target height;

(10) The quantity of active ingredient(s) or acid equivalent collected or detected at each sampling point in terms of kilograms per hectare (pounds per acre); and

(11) Where droplet size distribution is determined during the field evaluation, refer to § 163.126–3(c) for the reporting requirements.

Appendix A to Subpart J

Test Protocols. The following test protocols have been developed to provide guidance in the performance of pesticide plant hazard evaluation testing:

(1) *Seed germination.* Surface-sterilized seeds are germinated in Petri dishes between sheets of sterile filter paper moistened with the chemical; or the surface-sterilized seeds are germinated in washed quartz sand that has been sprayed or otherwise treated with a known quantity of the chemical.

Use at least ten seeds per dish. The seeds are incubated for up to 14 days. The seeds are observed weekly (or more frequently), and seed germination is reported as the number of emerged or germinated seeds.

The methods of Horowitz (1966), Kratky and Warren (1971), or Rubinstein et al. (1975) may be used in this assessment.

Recommended References

Horowitz, M. 1966. A rapid bioassay for PEBC, and its application in volatilization and absorption studies. *Weed Res.* 6:22–36.

Kratky, B.A., and G.F. Warren. 1971. The use of three simple, rapid bioassays on forty-two herbicides. *Weed Res.* 11:257–262.

Rubenstein, R., E. Cuirle, and H. Cole. 1975. Test methods for assessing the effects of chemicals on plants. EPA Report 560/5–75–008. NTIS PB 248–298.

(2) *Vegetative vigor-foliar spray.* The foliar spray can be applied by any commonly acceptable method using laboratory-, greenhouse-, or field-grown plants. The plants are to be sprayed during their most susceptible growth or reproductive stage as determined by prior testing. For vegetative vigor testing, the plant may be 0 to 4 weeks post-emergent (7–9 weeks for *Rhizobium*-inoculated soybeans). Types of sprays and methods of foliar applications may be found in the references below. Detrimental effects are to be reported as phytotoxicity, abnormal changes in growth and development, and/or abnormal changes in plant morphology.

Recommended References

Rubinstein, R., E. Cuirle, and H. Cole. 1975. Test methods for assessing the effects of chemicals on plants. EPA report 560/5–75–008. NTIS PB 248–298.

Truelove, B., (ed). 1977. Research methods in weed science. Southern Weed Science Society. Auburn Printing Inc., Auburn, AL. 221 pp.

(3) *Lemna gibba: Growth conditions.* The following are acceptable culture conditions for the growth and maintenance of *Lemna gibba* G3.

Species: *Lemna gibba* G3.
Environmental Conditions:
Light Intensity: 10 klux (10 k lum n /sq. meter)
Light Quality: warm white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 25° C
Culture Conditions:
Liquid culture
Nutrients: M type Hoagland's medium *without* EDTA or sucrose (Hillman, 1961 a & b); pH 5.0± 0.1 after autoclaving.

The vessel-to-medium ratio shall be 5 to 2. Maintain the *Lemna* stock under axenic conditions. The tests may be performed under non-axenic conditions as long as non-organic mediums are used.

Recommended References

Hillman, W.S. 1961a. Experimental control of flowering in *Lemna* III. A relationship between medium composition and the opposite photoperiodic responses of *L. perpusyilla* 6746 and *L. gibba* G3. *Amer. J. Bot.* 48:413–419.

Hillman, W.S. 1961b. The *Lemnaceae*, or duckweeds. *Bot. Rev.* 27:221–287.

(4) *Selenastrum capricornutum: Growth conditions.* The following are acceptable culture conditions for the growth and maintenance of *Selenastrum capricornutum.*

Species: *Selenastrum capricornutum* Printz.
Source: EPA Corvallis Laboratory, Corvallis, OR 97330.

Environmental conditions:
Light Intensity: 400 ft-c (4300 lumens/sq. meter)
Light Quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 24 ± 2°C
Culture conditions:
Liquid culture
Nutrients: U.S. EPA (1978) medium
pH 7.5

Recommended References

Environmental Protection Agency, National Eutrophication Research Program, 1971. Algal Assay Procedure: Bottle Test (AAP:BT), National Environmental Research Center, Corvallis, OR 97330.
Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The *Selenastrum Capricornutum* Printz algal assay bottle test. U.S. Environmental Protection Agency, Corvallis, OR 97330 (EPA 600/9–78–018).

(5) *Skeletonema costatum: Growth coinditions.* The following are acceptable culture conditions for the growth and maintenance of *Skeletonema costatum.*

Species: *Skeletonema costatum.*

Environmental conditions:
Light Intensity: 6–6.5 klux (6–6.5 k lumens/sq. meter)
Light Quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: 18° C continuous
Culture conditions:
Liquid culture
Nutrients: U.S. EPA (1974) medium
pH 8

Recommended References

U.S. Eutrophication and Lake Restoration Branch National Environmental Research Center-Corvallis. 1974. Marine Algal Assay Procedure: Bottle Test.
U.S. Environmental Protection Agency. 1978. Bioassy procedures for the ocean disposal permit program. U.S. EPA Laboratory, Gulf Breeze, FL 32561 (EPA–600/9–78–010).

(6) *A Freshwater Diatom Growth conditions.* The following are acceptable culture conditions for the growth and maintenance of *Navicula seminulum* or other selected freshwater diatom.

Species: (To be selected).

Environmental conditions:
Light Intensity: 400 ft-c (4300 lumens/sq. meter)
Light Quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 24 ± 2° C.
Culture conditions:
Liquid culture
Nutrients: U.S. EPA (1971) medium
pH 7.5

Recommended Reference

Environmental Protection Agency, National Eutrophication Research Program. 1971. Algal Assay Procedure: Bottle Test (AAP:BT). National Environmental Research Center, Corvallis, OR 97330.

(7) *Anabaena flos-aquae: Growth conditions.* The following are acceptable culture conditions for the growth and maintenance of *Anabaena flos-aquae.*

Species: *Anabaena flos-aquae* (Lyngb.) DeBrebisson.

Source: EPA Corvallis Laboratory, Corvallis, OR 97330.

Environmental conditions:
Light Intensity: 200 ft-c (2150 lumens/sq. meter)
Light Quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 24 ± 2° C
Culture conditions:
Liquid culture
Nutrients: U.S. EPA (1978) medium
pH 7.5 (not greater than 8.5)

Recommended References

Environmental Protection Agency, National Eutrophication Research Program. 1971. Algal Assay Procedure: Bottle Test. (AAP:BT). National Environmental Research Center, Corvallis, OR 97330.
Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. U.S. Environmental Protection Agency, Corvallis, OR 97330 (EPA 600/9–78–018).

(8) *Mutagenicity study procedures. Tradescantia reflexa* will be used as the tier 1 test species. The growth conditions are provided herein. The protocol to test for mutagenicity effects are given in Sawamura (1964), Sawamura and Jackson (1968) and Wada (1952).

Species: *Tradescantia reflexa* (tetraploid species).

Environmental conditions:
Light Intensity: 20 klux (20 k lumens/sq. meter)
Light Quality: incandescent and cool white fluorescent
Photoperiod: 16 hrs/8 hrs D/N
Thermoperiod: 24°C/18°C D/N
Relative Humidity: 60–70%
Culture conditions:
Soil (Sawamura, 1964 and Wada, 1952)
pH 6.0 (5.0–7.0)
For mutagenicity effects testing, ensure that the variety and ploidy levels are those specified in the references. Growth conditions and test protocols are provided in Sawamura (1964), Sawamura and Jackson (1968), and Wada (1962). Other protocols and plant species may be used with prior approval of the Agency.

Recommended References

Sawamura, S. 1964. Cytological studies on the effects of herbicides on plan cells *in vivo.* I. Hormonic herbicides. *Cytologia* 29:86–102.
Sawamura, S., and W. T. Jackson. 1968. Cytological studies *in vivo* of picloram, pyriclor, trifluralin, 2,3,6–TBA, 2,3,5, 6–TBA and nitralin. *Cytologia* 33:545–554.
Wada, B. 1952. Effects of chemicals on mitosis studied in *Tradescantia* cells *in vivo.* I. p-Acetyl aminotropollene. *Cytologia* 17:14–34.

(9) *Nitrogen fixation study procedures*—(i) *General.* When required, the effect of the pesticide on nitrogen fixation shall be determined by the acetylene reduction method. The following test conditions are common to all of the species to be tested.

(A) The chemical to be tested should be applied to the experimental medium for a period of time before and then to the incubation medium during the enzyme assay.

(B) Acetylene reduction will be allowed to occur for the required incubation time (30 to 60 min) under the environmental conditions in which the plant is grown. The acetylene partial pressure (PC_2H_2) will be 0.1 atm (10%).

(C) After the required incubation time, a sample of the vapor phase is obtained and either stored or assayed directly.

(D) Assays for ethylene content (acetylene reduction end-product) will be performed using a gas chromatograph equipped with a hydrogen ion flame ionization detector (F.I.D.). The gases will be separated on a column 3.2 mm wide (OD) 2 m long containing 80/120 mesh Parapak N or similar packing material. The standard curves for the ethylene standard must be provided with the retention times. Oven and injection temperatures and carrier gas used are at the discretion of the reseacher but must be reported.

(ii) *Protocols.* The following protocols are provided for guidance in the growth and maintenance of the species to be used for testing nitrogen fixation potential.

(A) *Legumes. Glycine max* is inoculated with *Rhizobium japonicum. Trifolium repens* is inoculated with *Rhizobium trifolii.* The general growth conditions and methods for determination of nitrogen fixation potential are provided below. Apply *Rhizobium* in a peat slurry to the seed. Use 7- to 9-week-old seedling for assays. Measure nitrogen fixation potential using nodules attached to roots. Note the number, size, and fresh weight of the nodules.

Environmental Conditions:
Light Intensity: 20–30 klux (20–30 k lumens/sq. meter)
Light Quality: fluorescent and incandescent
Photoperiod: 16 hrs/8 hrs D/N
Thermoperiod: 30/20°C D/N
Relative Humidity: 60%
Culture Conditions:
Sand/Nutrient culture
Nutrients: *Trifolium*—Crone's nitrogen-free medium.

Glycine—Sloger's nitrogen-free medium.
pH: 6–7 (not less than 5.2)

Recommended References

Allen, N. O. 1957. Experiments in soil bacteriology. pp. 93–94. Burgess Publ. Co., Minneapolis.
Masterson, C. L., and P. M. Murphy. 1976. Application of the acetylene reduction technique to the study of nitrogen fixation by white clover in the field. Pp. 299–316 *in* Symbiotic Nitrogen Fixation in Plants. P. S. Nutman (ed.). (IBP Vol. 7) Cambridge University Press, Cambridge, U.K.
Sloger, C. 1969. Symbiotic effectiveness and N_2 fixation in nodulated soybean. *Plant Physiol.* 44:1666–1668.

(B) *Azolla-Anabaena azollae.* Three species of *Azolla* occur in the continental United States: *A. caroliniana, A. mexicana,* and *A. filiculoides.* The growth, maintenance, and determination of nitrogen fixation potential by the symbiosis are found in the following references.

Recommended References

(i) *A. caroliniana.*

Peters, G. A., and B. C. Mayne. 1974. The *Azolla, Anabaena azollae* relationship II. *Plant Physiol.* 53:820–824.

Peters, G. A. 1975. Studies on the *Azolla-Anabaena azollae* symbiosis. Pp. 592–610 *in* Proc. Internat. Symp. on Nitrogen Fixation. W. E. Newton and C. J. Nyman, eds. Washington State Univ. Press: Pullman, WA.

(ii) *A. mexicana.*

Holst, R. W., and J. H. Yopp. 1979. Environmental regulation of nitrogenase and nitrate reductase as systems of nitrogen assimilation the *Azolla mexicana-Anabaena azollae* symbiosis. *Aquatic Bot.* 7:369–384.

Holst, R. W., and J. H. Yopp. 1979. Studies of the *Azolla-Anabaena* symbiosis using *Azolla mexicana.* I. Growth in nature and laboratory. *Amer. Fern J.* 69:17–25.

(iii) *A. filiculoides.*

[No specific references are available. Refer to the publications in (i) and (ii) (above)], and

Moore, A. W. 1969. *Azolla:* biology and agronomic significance. *Bot. Rev.* 35:17–34.

(C) *Anabaena.* Either *A. flos-aquae* or *A. cylindrica* may be used. The growth conditions for *A. flos-aquae* are given in paragraph (7) of this Appendix. Those for *A. cylindrica* are given in Hattori (1962). In both cases, the alga must be grown on a nitrogen-free medium. Nitrogen fixation determination must be performed utilizing intact filaments and cells. The technique of Stewart et al. (1971) shall be used and the samples assayed without termination of the reaction with trichloracetic acid.

Recommended References

Hattori, A. 1962. Light-induced reduction of nitrate, nitrite, and hydroxylamine in a blue-green alga, *Anabaena cylindrica. Plant Cell Physiol.* 3:355–369.

Stewart, W. D. P., T. Mague, G. P. Fitzgerald, and R. H. Burris. 1971. Nitrogenase activity in Wisconsin lakes of differing degrees of eutrophication. *New Phytol.* 70:497–509.

(D) *Azotobacter vinelandii. Azotobacteri vinelandii* can be grown on a modified Burke's medium supplemented with 1 μg Mo/liter as $Na_2Mo0_4.2H_20$ (Burns et al. 1971). Nitrogen fixation potential is determined using either the method of Balandreau and Dommergues (1973) or that used for *Anabaena* as provided in section (C) above.

Recommended References

Burns, R. C., W. H. Fuchsman, and R. W. F. Hardy. 1971. Nitrogenase from vanadium-grown *Azotobacter:* Isolation, characteristics, and mechanistic implications. *Biochem. Biophys. Res. Comm.* 42:353–358.

Balandreau, J., and Y. Dommergues. 1973. Assaying nitrogenase (C_2H_2) activity in the field. *Bull. Ecol. Res. Comm.* 17:247–254. (Now: Ecol. Bull. Sweden Nat. Res. Council).

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